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Critical Review

Polychlorinated Biphenyl Tissue-Concentration Thresholds for Survival, Growth, and Reproduction in Fish

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Abstract: Polychlorinated biphenyls (PCBs) have left a legacy of environmental contamination. Even though they were banned from production and active use in the 1970s, they persist in the environment and still have the potential to impact aquatic life. Our objective was to identify data from controlled laboratory studies of PCB-related adverse effects in fish and to conduct a meta-analysis on mortality, growth, and reproductive (MGR) threshold responses. For each endpoint type, we compiled data on the lowest-observed-adverse effect concentration (LOAEC) and the degree of effect at the LOAEC as a percentage of control. The LOAECs were expressed as tissue concentrations, so the term lowest-observed-adverse-effect residue concentration (LOAER) was used to represent PCB exposures. The lower limit of applicability was set at 0.1 μg/g total PCB tissue concentration, below which adverse MGR effects in fish were not supported by the data. Sensitivity distributions identifying the probability of adverse effects in fish populations or communities predicted that 25% of fish species would be impacted between 0.1 and 7.5 μg/g. Concentration–response threshold regressions were developed from the MGR datasets. For example, a 1 μg/g total PCB tissue concentration would predict effects of 17% mortality, 15% growth, and 39% reproductive. The analysis determined the degree of adverse response, with uncertainty estimates, expected across a broad range of PCB tissue exposure concentrations in fish. Data generated from MGR endpoints were combined to determine an approach for overall effect thresholds for PCB-related injury in fish. The MGR datasets included only laboratory data; however, responses were compared with field-observed effects. The present review provides a comprehensive assessment of PCB-induced injury in fish utilizing a data-inclusive approach. *Environ Toxicol Chem* 2019;38:712–736. Published 2018 Wiley Periodicals Inc. on behalf of SETAC. This article is a US government work and, as such, is in the public domain in the United States of America.

Keywords: Polychlorinated biphenyl; Fish; Tissue-residue effects; Injury assessment

INTRODUCTION

Polychlorinated biphenyls (PCBs) are organic chemicals that have a biphenyl structure and varying degrees of chlorination (Figure 1). They were used in many industrial applications because of their properties of resistance to degradation, high flash point, and high heat capacity. These chemical properties made PCBs ideal for use in electrical components, including heat transfer fluids, transformers, and capacitors. Industrial applications of PCBs grew during the mid-20th century, with production in the United States reaching 32 million pounds by 1957 and nearly 80 million pounds by 1980 (Cairns et al. 1986). Manufacture of PCBs in the United States was under the trade name Aroclor®; they were manufactured and marketed in Europe and Asia under the trade names Clophen® and Kanachlor®, respectively. Synthesis of PCBs occurred by bubbling chlorine gas through a solution of biphenyl, resulting in mixtures that varied in congener composition and were classified according to the degree of chlorine substitution on the biphenyl ring. Mixtures of PCBs were marketed and sold based on the percentage of chlorine content. For example, Aroclor 1242 contained 42% chlorine by weight, and Aroclor 1254 contained 54% chlorine by weight. Each of the commercial mixtures of PCBs sold in the United States had different combinations of the possible 209 individual PCB congeners.

The recognition that PCBs were of environmental concern became widespread after Jensen (1966) published the first description of PCB contamination in an aquatic food web. Jensen observed trophic-level magnification of PCB concentrations in Baltic salmon (*Salmo salar*) and white-tailed eagles (*Haliaeetus albicilla*) in the Baltic Sea (Jensen 1966, 1972). Over the next 5 decades it became evident that environmental contamination by PCBs was a global issue. Numerous reviews have summarized the persistence, movement, and bioaccumulation properties of PCBs in the environment (e.g., Waid 1986;

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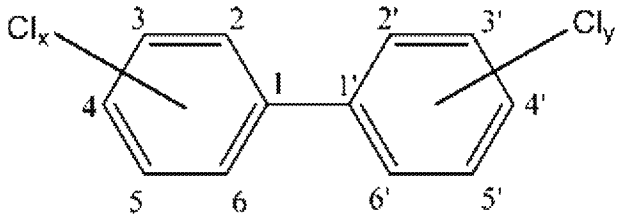

FIGURE 1: General chemical structure of polychlorinated biphenyls.

Tanabe 1988; Voogt et al. 1990). Based on persistence in the environment, PCBs were included in the United Nations Environmental Programme Stockholm Convention (2001) list of persistent organic pollutants (POPs) and later in 2004 by the Organisation for Economic Co-operation and Development. The goals of these designations were to have global agreement on the management and phase-out of the listed POPs, including PCBs. Even though PCBs had been banned from production in the United States in 1979, PCB-containing electrical transformers and capacitors in use at that time were approved to remain in-place or "grandfathered" into continued use. It was estimated that approximately 96% of all manufactured PCBs worldwide (~1.2 million tons) were either in the environment (31% of total production) or landstocked (65% of total production) in continued use or unsecured landfills (Tanabe 1988). Thus, the potential for continued release of PCBs into both aquatic and terrestrial environments continued over the next decades.

Environmental contamination concerns increased through the end of the 20th century as more information came to light on the toxicity of PCBs to humans and ecosystems. The PCBs were designated as human carcinogens and a better understanding of the different potencies of individual PCB congeners was formed during this time (e.g., Safe et al. 1985; Safe 1994, 1990). Congeners were observed to have different toxicities and modes of action, based on the chlorination pattern on the biphenyl rings (Safe et al. 1985; Safe 1994, 1990). A small set of the PCB congeners (~18 congeners) with lateral chlorines and non-*ortho*-chloro-substitution or mono-*ortho*-chloro-substitution patterns were found to bind to the aryl hydrocarbon receptor (AhR) and elicit dioxin-like toxicity (summarized in Van den Berg et al. 1998). All vertebrates possess homologous forms of the AhR and have varying degrees of sensitivity to dioxins and dioxin-like PCBs. Other modes of action of PCBs include neurotoxicity, endocrine disruption, and carcinogenicity (Safe et al. 1985; Safe 1994, 1990). Toxicity thresholds for commercial PCB mixtures and individual PCB congeners in wildlife have been developed over the past 20 yr (Giesy and Kannan 1998; Kannan et al. 2000; Su et al. 2014).

The goal of the present study was to evaluate PCB-induced adverse effects data in fish and to develop tissue concentration–based effects thresholds. Assessment of the risk posed to fish populations by PCB exposure has been essential for natural resources management. Federal programs, such as the Natural Resource Damage Assessment and Restoration (NRDAR) Program administered under the Department of the Interior and the National Oceanic and Atmospheric Administration or the Superfund Program administered by the US Environmental Protection Agency, routinely evaluate contaminant-related effects using metrics of survival, growth, and reproduction. Thus, our review and analysis focused on survival, growth, and reproduction, effects designated as injuries in prospective and retrospective risk assessments. We accomplished this aim in 2 steps: first, a comprehensive review of the fish PCB effects literature; and second, development of a data-inclusive approach for analysis of PCB exposure–effect/injury relationships across all fish species tested.

MATERIALS AND METHODS

Data identification

Literature review. A comprehensive literature search was conducted to initiate this analysis on the effects of PCBs on fish. We focused on the endpoints of mortality, growth, and reproduction (MGR). Multiple databases were searched including Google Scholar (Google), Web of Science (Thomson Reuters), and SCOPUS (Elsevier). We excluded studies that focused on human health or other nonfish species, as well as studies that evaluated only PCB exposure and bioaccumulation. The primary literature was the preferred source of all data; however, previous reviews on the effects of PCBs on fish (Monosson 2000; Meador et al. 2002) gave critical insights into data selection and evaluation and provided a check to ensure that critical studies were included in the present meta-analysis.

Data inclusion criteria. Six criteria were developed to identify appropriate data for our meta-analysis (Figure 2), based on standards for determination of injury under the NRDAR framework (as laid out in 43 CFR Part 11.62; US Department of the Interior 2009) and the guidelines set forth by Rosenberger (1987).

The first criterion required studies to evaluate MGR effects. The MGR endpoints provide practical and well-defined data that are useful in decision making (US Environmental Protection Agency 2003). Increased mortality and decreased reproductive capacity are clearly deleterious to fish populations and are considered injurious to fish (see 43 CFR Part 11.62; US Department of the Interior 2009). Growth impairment (reduction) in fish also has a substantial impact on populations, because smaller fish are more susceptible to loss through predation, do not compete for resources as well, and can indicate reduced fitness of the population (Groh et al. 2015).

The second criterion required that the effects be unequivocally attributable to PCB exposure. For inclusion, studies must have shown that fish exposed to PCBs exhibited adverse effects relative to a control group, thus restricting our primary analysis to laboratory studies.

The third criterion required that fish be exposed to PCB mixtures, as occurs in the environment. Although all PCBs are structurally similar, the number and orientation of chlorines influence toxicity, both empirically and mechanistically (Safe et al. 1985; Safe 1990, 1994). Laboratory studies of individual congeners, although mechanistically informative, do not

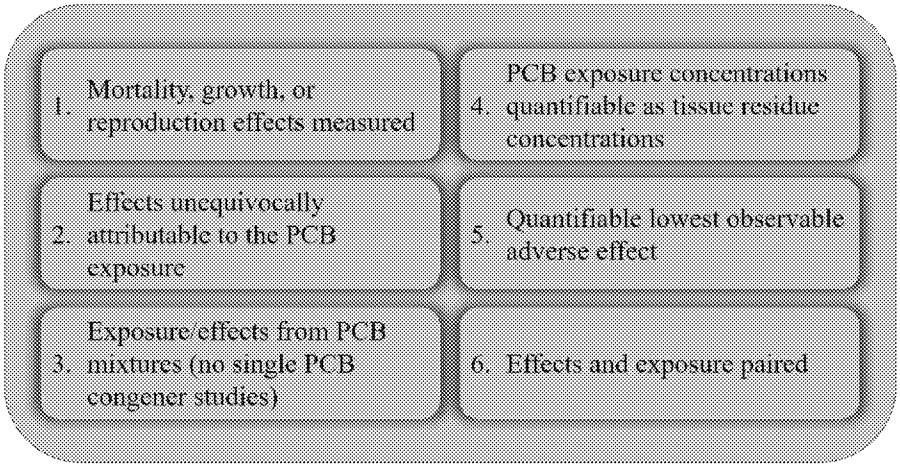

FIGURE 2: Data selection criteria for meta-analysis of polychlorinated biphenyls (PCBs) in fish.

represent how PCBs are likely to be encountered in the environment. Moreover, data from single PCB congener studies are not readily translated from a laboratory study to a field exposure (Giesy and Kannan 1998). In addition, studies that only included the most potent dioxin-like PCBs (PCBs 77, 81, 126, and 169; Giesy and Kannan 1998) were excluded. Fish toxicity studies using only dioxin-like PCBs ignore the potential for impacts from other PCB modes of action. To facilitate the use of multiple PCB mixtures, the dose metric of total PCBs was used.

Criterion 4 required that PCB exposure concentrations be represented as quantifiable tissue residue concentrations. It is well established that PCBs bioaccumulate in tissues (Veith et al. 1983, 1979; Safe 1994; Meador et al. 2011), and the use of a tissue residue approach as an exposure metric provides the most direct connection to the observed effects (McElroy et al. 2011). This criterion required studies to measure PCBs in exposed fishes or provide sufficient data to allow tissue concentrations to be calculated. Quantification and conversion steps for estimation of PCB tissue residues are described in detail in the *PCB exposure concentration selection and translations* section).

Fifth, PCB-induced effects must be quantifiable for inclusion in this analysis. This criterion required that biological responses in PCB-exposed fish be measurably different from control or lower exposure organisms. Ideal data presented exposed fish effects that were statistically different from effects in control fish. This approach provided scientifically defensible determination of effects. In each case, the lowest-observed-effect concentration was selected for inclusion. Within this analysis, the effect terminology used was lowest-observed-adverse-effect residue concentration (LOAER), to more accurately reflect the type of data being collected (further described in the *Data selection and translation* section). The LOAER provided a definitive measure of adverse outcome from each study, that is, a single concentration for which statistically significant, PCB-related toxic effects were observed, presumably within the linear portion of the dose–response curve.

The sixth criterion required that the LOAER effect levels be paired with an exposure concentration to provide a single point for comparison across studies.

Data selection and translation. Data from studies meeting the 6 selection criteria were compiled. The primary data extracted from each study were individual LOAERs for MGR and the PCB tissue concentrations at which each of those effects occurred. Each paired set of primary data (tissue concentration +LOAER expressed as percentage effect) was referred to as a datapoint, within the context of the present review. Ancillary data were also collected for each datapoint, including PCB mixture type, fish species, and fish life history information (specifically temperature, salinity, and fish life stage). Other study data collected included route and duration of PCB exposure. All extracted data were converted to standardized units (e.g., μg/g for PCB tissue concentrations) to facilitate metadata analysis. Conversions for each type of data followed set procedures (described in the *Effect data selection and translation* section). Original and converted values specific to each datapoint are given in the Supplemental Data (Tables S1–S3). Abbreviations and information for each mixture are also given in Supplemental Data (SI Table PCB).

Effect data selection and translation. The MGR data were quantified using effect-specific endpoints. Mortality effects were defined by changes in survival between PCB-exposed and control groups. Studies with measurements of hatchability and larval survival were included among mortality studies. Hatchability compared the number of surviving, viable larval fish with the initial number of eggs. Growth effects were defined by change in weight or length in PCB-exposed treatments relative to control groups. Only reduced growth data (i.e., fish at LOAER significantly smaller than control fish) were included in our analysis, because decreased growth is generally accepted as an adverse effect. Growth data were excluded in cases in which size-selective mortality, (i.e., smaller fish dying first) may have influenced growth calculations (Seelye and Mac 1981; Garrido et al. 2015). For reproduction effects, only reproductive impairment in adult fish was

included in the analysis under this category. Often studies on reproduction reported effects on eggs or larval fish (i.e., hatchability, larval survival; Hansen et al. 1973; Schimmel et al. 1974; Freeman and Idler 1975; Hugla and Thome 1999); those endpoints were more accurately characterized as mortality responses and were included in the mortality dataset. For adult fish, reproductive endpoints included egg production (fecundity), fertilization rate, and number of spawns.

The MGR effects at the LOAER were normalized to percentage effect relative to control responses to provide for comparisons across studies (Equation 1). The LOAER and control effects were expressed as responses that were reduced because of toxicity (e.g., % survival, body weight, number of eggs produced). This provided consistency across levels of effect, particularly for mortality endpoints, for which responses were commonly either %mortality or %survival. Any effect orientation changes are given in the Supplemental Data, Tables S1 to S3 . Furthermore, Equation 1 oriented all data so that percentage effect would increase with PCB tissue concentrations (e.g mortality, growth inhibition, and reproductive impairment). Normalization helped identify the actual impact of PCBs, beyond the natural variation in MGR. A small number of studies had quantifiable differences in mortality between exposed and control fish, although statistics were not reported. These studies were included if the percentage effect was >50%. This allowed the inclusion of more data, while reducing the chance that differences between the control and exposed groups were not real. These cases are identified in the Supplemental Data (Tables S1–S3).

$$\text{Percentage effect conversion: } 1 - \frac{\text{LOAER response}}{\text{control response}} = \%\text{effect} \quad (1)$$

PCB exposure concentration selection and translations. Concentration data at the LOAER were most commonly reported as micrograms (μg) of total PCBs/gram (g) of whole-body wet weight. The LOAER concentration data were standardized to this reporting metric using the protocols described below.

The most common translation was from dietary dose to whole-body wet weight. A 50% conversion from measured PCB dietary dose to whole-body wet weight was utilized (Equation 2). Previous work established 50% assimilation of PCBs at dietary dose concentrations <200 μg/g, with approximately 25% assimilation efficiency at greater exposure concentrations (Opperhuizen and Schrap 1988). Findings in other studies were similar (Gruger et al. 1975, 1976; Madenjian et al. 1999). Meador et al. (2002) also utilized a 50% assimilation efficiency estimate for exposure in their examination of PCB-induced effects on salmonid species (Meador et al. 2002). In Equation 2, *x*, the concentration of PCBs (μg) in food (g wet wt) was multiplied by the assimilation factor (0.5) to estimate *y*, the concentration of PCBs (μg)/g of whole-body wet weight.

$$\text{PCB in diet conversion: } x\ \mu\text{g/g PCBs in diet} \times 0.5 = y\ \mu\text{g/g PCBs in tissue} \quad (2)$$

A few studies reported study-specific bioconcentration factors (BCFs) instead of directly reporting PCB tissue concentrations. For example, Veith et al. calculated BCF values from PCB tissue concentrations and the aqueous (exposure) PCB concentrations, but did not actually report the tissue concentrations (Veith et al. 1979). The reported BCF and aqueous concentrations in these cases were used to determine whole-body tissue concentrations (Equation 3). Study-specific adjustments to Equation 3 are provided in the Supplemental Data (Tables S1–S3). In Equation 3, the study-reported BCF was multiplied by *x*, the concentration of PCBs in water, to estimate *y*, the concentration of PCBs (μg)/g of whole-body wet weight.

$$\text{BCF conversion: BCF(reported)} \times x\ \text{mg/L PCBs in water} = y\ \mu\text{g/g PCBs in tissue} \quad (3)$$

One study included in the present analysis (Black et al. 1998a) used intraperitoneal injection for dosing and required translation of injected dose to estimate whole-body wet weight tissue concentration. Evidence supported a 75% assimilation of measured injected PCB dose to total PCB wet weight (Equation 4; reviewed by Meador et al. 2002). In Equation 4, *x*, the concentration of PCBs (μg) injected/g body wet weight was multiplied by the assimilation factor (0.75) to estimate *y*, the concentration of PCBs (μg)/g of whole-body wet weight.

$$\text{PCB injection conversion: } x\ \mu\text{g/g PCBs injected} \times 0.75 = y\ \mu\text{g/g PCBs in tissue} \quad (4)$$

The PCB measurements reported as dry weight were converted to wet weight based on an assumption of 80% moisture content for fish (dry wt 20% of wet wt; Equation 5; Meador et al. 2002). Lipid normalized concentrations were translated to wet weight assuming 4.6% lipid content in fish (Equation 6). If study or species-specific lipid concentrations were reported/available, they were used.

$$\text{Dry weight conversion: } x\ \mu\text{g/g dry weight} \times 0.20 = y\ \mu\text{g/g wet weight} \quad (5)$$

$$\text{Lipid weight conversion: } x\ \mu\text{g/g lipid weight} \times 0.046 = y\ \text{PCB } \mu\text{g/g wet weight} \quad (6)$$

Total PCBs were used as the metric for data standardization. Most included studies used total PCBs to report concentrations. Total PCBs were also often used in injury assessment or risk assessment cases (e.g., DeVault et al. 2001; Blazer et al. 2006). The detailed congener information required to calculate toxic equivalency factor (TEQ)-based exposure estimates were not

available for most of the studies. An extensive evaluation of total PCBs versus TEQs in terms of concentration–response (see the Supplemental Data, total PCB vs TEQ) suggested that total PCBs provided the best approach for the development of PCB injury thresholds for this dataset.

Data analysis

Sensitivity distribution development. Probabilistic sensitivity distributions were developed separately for the MGR LOAER datasets. Probabilistic sensitivity distributions, similar to chemical toxicity distributions and species sensitivity distributions (SSDs), were applied to our current datasets to develop a probabilistic injury assessment for PCBs. This allowed us to identify the percentage of species expected to display significant mortality, growth, or reproductive impact at any given total PCB concentration. The use of LOAERs anchored these distributions to measured effect concentrations. We recognize that the included species and PCB mixtures may not represent the full range of sensitivities. This approach simply represents the best available information we have at this time, which can be updated with new information as it becomes available.

The development of the probabilistic injury distributions followed a modified SSD approach (Berninger and Brooks 2010). Tissue concentration datasets were log-transformed and tested for normality. Each PCB LOAER concentration within a dataset (n = # points within dataset) was assigned a numeric rank (i) from low to high. Ranks were then converted to a cumulative probability (percentage rank [j]) using the Weibull equation (Equation 7). The PCB tissue concentration–percentage rank pairs were used to generate a probability-log scale linear regression for the MGR datasets. These probability regressions estimated the number of species experiencing significant effects at any given concentration (previously described in depth by Solomon and Takacs 2001; Berninger and Brooks 2010; Berninger et al. 2016, 2011). The slope (m) and intercept (b) of each regression line were used to identify the percentage of species affected (centile [C]) or a tissue concentration value (x), using NORMSDIST and NORMSINV functions in Microsoft Excel® (Equations 8 and 9). Probabilistic distribution regressions were used to determine thresholds of effect at specific PCB tissue concentrations across multiple orders of magnitude (Equation 8) and to determine concentrations associated with a specified percentage of species effected (5–95%). Distributions were developed for mortality, growth, and reproduction independently.

$$\text{Weibull equation: } j = (i \times 100)/(n+1) \quad (7)$$

$$\text{Probability estimate: } C = \text{NORMSDIST}(m \times \log_{10}[x] + b) \quad (8)$$

$$\text{Concentration estimate: } x = 10^{([\text{NORMSINV}(C)-b]/m)} \quad (9)$$

PCB concentration: Effect regressions. The next step in the meta-analysis was the direct evaluation of the relationship between PCB tissue concentrations and effects. Log-transformed concentrations were compared with their associated percentage effects at the LOAER using linear regression. Given the range of LOAERs across studies, this produced PCB concentration–effect relationships across a wide range of potential concentrations. Each dataset (MGR) was evaluated to determine whether it fit the statistical assumptions required for linear regression, including normality and homogeneity of variance. Values with studentized-deleted residuals > 3 were identified as potential outliers, and specifically evaluated further. Regression calculations, statistics, and graphing were conducted in SigmaPlot (Ver 13.1; Systat Software).

Regression analysis was conducted on raw and condensed concentration–effect datasets. The raw data on effects were highly variable, so analysis on condensed data was conducted to reduce variability and produce regression analyses with greater predictive power, as is common practice within this type of meta-analysis (Monosson 2000; Meador et al. 2002). The LOAER tissue concentrations were divided into 5 groups (20th percentiles or quintiles), which provided better resolution and could also be applied across all MGR datasets without resulting in empty groups (null sets). The PERCENTILE function (Microsoft Excel) was used to identify 20th, 40th, 60th, and 80th percentile concentrations from the entire dataset, which were subsequently used to define quintile boundary concentrations for individual mortality, growth, or reproductive datasets for consistency (Equation 10). The central tendency of tissue LOAER concentrations within each quintile (0–20, >20–40, >40–60, >60–80, >80–100) was calculated as the geometric mean. Standard error of quintile means was calculated according to Equation 11. All central tendency values for log-transformed LOAERs were calculated as geometric means and referred to as geomean(s). Arithmetic means (averages) and standard errors (SEs) for percentage effect were calculated for each quintile. Normality of quintile values was assessed as above.

$$\text{Percentile: } x\ \mu\text{g/g} = \text{PERCENTILE}([\text{select-all dataset } \textit{PCB concentrations}], y) \quad (10)$$

The quintile percentiles (y; 0.2, 0.4, 0.6, 0.8) identify concentrations (x) that mark the bounds of each quintile.

$$\text{Standard error of geometric mean} = \text{qG} \times (\text{StDev}[\log \text{qX}]/\text{SqRt}[n-1]) \quad (11)$$

where the geometric mean of a quintile qG = quintile geometric mean; qX = standard deviation of the log values in that quintile; and n = number of values within the quintile.

Linear regressions along with applicability statistics were regenerated using the quintile data from each dataset (MGR). For all the regressions, the significance ($p < 0.05$) and the coefficient

of determination (r^2) were calculated by comparing percentage effect and log PCB tissue concentrations (LOAER values).

PCB concentration: Effect regression threshold calculation and prediction development. The MGR quintile regressions provided slope and intercept information that was then used to generate concentration–effect regression thresholds. Percentage effect can then be estimated at any given PCB exposure concentration (Equation 12), and PCB concentrations estimated for any given percentage effect (Equation 13). Effect estimates were calculated in a standard spreadsheet program to simplify the process and provide a standardized approach that can be applied in a variety of regulatory and decision-making scenarios. The MGR percentage effects could be estimated across order of magnitude ranges of PCB tissue exposure concentrations and PCB tissue concentrations calculated for percentage effects from 5 to 95%.

Linear regression percentage effect estimate:

$$y = (m \times \log_{10}[x] + b) \quad (12)$$

Linear regression concentration estimate: $x = 10^{(y-b)/m}$ (13)

Applicability limits within our analysis were defined based on the input data. We established a lower cutoff of 0.1 μg/g for the subsequent analysis of LOAER values. The lowest LOAERs within the MGR datasets occurred in the range of 0.1 μg/g, but not below. Furthermore, background PCB concentrations, at and below the 0.1 μg/g level, were observed in control fish without reported MGR effects. The PCB concentrations below this 0.1 μg/g cutoff are not expected to elicit adverse MGR effects based on our review of the literature. The upper limit for prediction of MGR effects based on PCB tissue concentration was set at 3000 μg/g, based on the average molecular weight of PCBs and the corresponding threshold for mortality from narcosis alone. In other words, if PCB concentrations were to reach this level in tissue, all fish would be expected to die from the nonspecific narcotic mode of action. This cutoff was more theoretical than practical because the data clearly showed that mortality occurred well below 3000 μg/g. Values above or below these limits were identified within tables and figures to denote that they were beyond practical applicability of the models.

PCB concentration: Effect threshold uncertainty analysis. Uncertainty regressions were developed for the MGR datasets. These were based on the assumption that each quintile percentage effect represented the central point of a normal distribution of percentage effects. To identify this distribution, an SE was multiplied by a probability factor, analogous to the approach used to calculate confidence intervals. Dataset SE was calculated as the average of the SEs for percentage effect at each quintile. The probability factor, the degree of normal variability around each percentage effect quintile, was determined using a 2-tailed *t* distribution. The *t* values were calculated at 50, 60, 70, 80, 90, 95, and 97.5% probability (Equation 14). Dataset SEs were multiplied by the *t* values at each probability level to establish dataset-specific uncertainty factors. The uncertainty factor was added to (and subtracted from) the quintile average percentage effect values to generate upper (and lower) uncertainty for each probability level. These percentage effect uncertainty sets (50, 60, 70, 80, 90, 95, and 97.5%) were regressed against the geometric mean quintile tissue concentrations to generate a series of uncertainty regressions. Using the slope and intercepts from these regressions (Equations 12 and 13) provided uncertainty ranges for the concentration–effects regressions.

$$t - \text{distribution value: } (x)t \text{ value} = \text{T.INV.2T}([1 - y], df) \quad (14)$$

The probability (y), in decimal percent, and degrees of freedom (df) were used to calculate a *t* value.

Evaluating testing parameters across individual studies. We compared ancillary information within datasets to test our assumption that including all available data provided robust and generally applicable estimates of PCB effects in fish. Previous injury assessments have often limited data selection based on species of concern, life history characteristics (e.g., temperature and salinity), or the mixture of PCBs deemed to be present in the geographic area. This limits the amount of data available to make assessments, often to only 1 or 2 studies. Although limited data prevented meaningful multivariate analysis, 5 ancillary variables were evaluated: PCB mixture, species, life stage, salinity tolerance, and temperature preference. Analysis of the PCB mixture was of particular interest given that 13 different PCB mixtures were included in our study and it has been well established that different mixtures of PCBs have different potencies (Tillitt et al. 1991; Harris et al. 1993). Twenty different fish species were included. The other ancillary data were evaluated as binary variables: life stage (early life stage [ELS] or juvenile/adult), salinity tolerance (fresh or saltwater), and temperature preference (cold or warm water).

Average percentage effect and geomean PCB tissue concentration, along with SEs, were calculated for each subgroup of ancillary variables within the MGR datasets. Differences among subgroups within each variable were evaluated using an analysis of variance (ANOVA) with post hoc testing. Data from the binary variables (life stage, salinity, and temperature) were used to generate linear regressions for each subgroup, and a concentration–effect regression was generated for each individual PCB mixture and fish species with sufficient data ($n \geq 3$).

Application of metadata analysis to evaluate cumulative effects. We developed the cumulative largest effect model (CLEM) to integrate simultaneous adverse MGR effects expected at a given exposure. The CLEM uses the most sensitive MGR effect at a given concentration. The CLEM additively combines mortality and growth given that fish exposed to PCBs experience mortality monotonically with concentration, whereas those that survive have reduced growth (Nebeker et al. 1974; DeFoe et al. 1978; Mauck et al. 1978; Bengtsson 1980; Mayer

et al. 1985; Thomas and Wofford 1993; Fisher et al. 1994; Black et al. 1998a; Orn et al. 1998; Gutjahr-Gobell et al. 1999). The largest cumulative effects, either reproduction (R) or the additive mortality and growth (M+G) effect rates, were used to generate the CLEM. The CLEM prevents double counting of any single effect. Furthermore, the reproductive dataset was used independently, based on the fact that reproductive effects of PCBs on fish generally occurred at lower concentrations than effects on growth and mortality, along with the idea that reproductive effects will generally occur through different pathways than mortality and growth-related effects. Moreover, reproductive effects limit populations through decreases in natality, whereas mortality and growth are ultimately measures of loss to the juvenile/adult within a population. Independent analysis of each of these adverse effects fails to consider the influence that each of these outcomes exerts on a population simultaneously. Therefore, we developed the CLEM for the evaluation of potential effects of PCB exposures on fish populations across a wide range of exposures, as well as through multiple, co-occurring mechanisms.

The CLEM calculations were derived from reproduction and mortality + growth dataset regressions. The mortality and growth regression percentage effect responses were added at designated concentration intervals (0.01, 0.1, 1, 10, 100, and 1000 μg/g). The CLEM concentration–response regression was then based on the most sensitive endpoint, either mortality + growth or reproduction percentage effects, at each designated exposure concentration. The CLEM regression identified cumulative concentration-effects across the range of expected PCB concentrations, following procedures previously described (Equations 12 and 13).

Analysis of field-derived PCB effects data

Field-derived PCB effects data in fish were compared with the laboratory-based data in an effort to corroborate the toxicity threshold estimates. Thus field data were vetted and compiled according to the same criteria developed for laboratory data, with the following caveats. Criteria 1, 3, 4, and 6 were used without modification. Effects evaluated from the field studies (i.e., mortality, growth, reproduction) were in concordance with laboratory effects data. The studies included measured tissue concentrations of PCB mixtures. Each study included both effects and concentration data. Criterion 2 (effects solely from PCBs) and criterion 5 (quantifiable lowest observable effect) required modification. The presence of non-PCB contaminants and abiotic/biotic differences at study locations make unequivocal PCB attribution of effects and establishing noneffected reference population effects difficult. Study data were included if PCBs were reported as the primary contaminant driver of effects. Percentage effect was determined on a case-by-case basis. The designated reference sites were not necessarily PCB free, and in some cases field data followed a gradient of exposure/responses. To establish percentage effect responses, exposed fish were compared with responses in fish from a designated reference site within the study, or in some cases between substantial and nonsubstantial effects, as identified by study authors (see details in the Supplemental Data, Field Data). For all field studies, information was collected on the specific field location, PCB mixture (if available), fish life history (species, temperature, salinity, and life stage), whole-body tissue concentrations, percentage effect, and specific endpoint measured.

Vetted individual and average field responses (geomean LOAER, average percentage effect) for MGR were compared with laboratory-generated LOAER regressions.

Statistics

Statistics were calculated using Microsoft Excel and SigmaPlot (Ver 13.1; Systat Software). Excel was used to calculate all individual data translations. This included means, geometric means, SEs, quintiles (both tissue concentration and percentage effect), and uncertainty factors. Normality, regression, and ANOVA were calculated using SigmaPlot. Significance was set at 95% ($p < 0.05$) for all analyses. All normality was evaluated using Kolmogorov–Smirnov with Lillifors correction. Each regression was evaluated to determine whether it fit the statistical assumptions required for linear regression, including normality, and homogeneity variance (measured as equal variance). For ANOVA, normality (Kolmogorov–Smirnov with Lillifors correction) and equal variance (Brown–Forsythe) were evaluated. If data did not meet normality requirements, an alternate ANOVA approach was used, Kruskal–Wallis one-way ANOVA on ranks, to provide the assessment. Post hoc testing approaches included Tukey's test for pairwise comparisons for data with normal distribution or Dunn's test for non-normal data in which ranks were evaluated.

RESULTS

The evaluation of the available literature on adverse effects of PCBs on fish found 31 laboratory MGR studies that fit the acceptability criteria. This resulted in 55 datapoints for LOAER, divided among mortality (30 datapoints; Table 1), growth (17 datapoints; Table 2), and reproduction (8 datapoints; Table 3). The 55 datapoints represented 16 PCB mixtures and 22 species. The individual MGR datasets also included varied species and PCB mixtures (Table 4). The different types of PCB mixtures evaluated (10 for mortality, 10 for growth, 7 for reproduction) represent most of the common commercial mixtures (see SI Table PCBs for PCB mixture descriptions and abbreviations). The number of fish species tested for PCB effects (mortality, 16; growth, 11; reproduction, 6) was relatively large, given the limited number of species usually used in effect-driven ecotoxicology. The MGR PCB tissue concentration data required log-transformation to meet conditions of normality. The geometric mean LOAER values (±SE) were 49.4 ± 8.9, 19.3 ± 4.2, and 9.8 ± 4.7 μg/g, for MGR, respectively. In addition, the 20th percentile quintile boundary concentrations were calculated (Table 4). Percentage effect values for the LOAERs were normally distributed for MGR. The mean percentage effects were 54.8 ± 4.4, 26.5 ± 3.8, and 50 ± 5.7%, for mortality, growth, and reproduction, respectively.

TABLE 1: Mortality-related lowest-observed-adverse-effect residue concentrations and other critical information from criteria-selected laboratory studies of fish exposed to polychlorinated biphenyls (PCBs)

PCB	Common name	Scientific name	Life stage	Temp.	Saline	Exp. (d)	Route	Concn. µg/g	Concn. code	Effect %	Quint	Reference
A1260	Barbel	*Barbus barbus*	ELS	Warm	Fresh	75	par	0.26	D	92	—[a]	Hugla and Thome 1999
A1254	Tilapia	*Oreochromis niloticus*	ELS	Warm	Fresh	15	di	0.75	Fd	57	Q1	Coimbra and Reis-Henriques 2007
A1254	Lake trout	*Salvelinus namaycush*	ELS	Cold	Fresh	176	aq/di	1.53	M	32	Q1	Berlin et al. 1981
A1254	Atlantic croaker	*Micropogonias undulates*	Adult	Warm	Salt	17	di	2.1	Fd	44	Q1	Thomas and Wofford 1993
PCB-ORN	Zebrafish	*Danio rerio*	Adult	Warm	Fresh	91	di	2.7	M	18	Q1	Orn et al. 1998
A1254	Sheepshead minnow	*Cyprinodon variegates*	ELS	Warm	Salt	28	par	5.1	m	22.	Q1	Hansen et al. 1973
A1254	Sheepshead minnow	*Cyprinodon variegates*	ELS	Warm	Salt	28	aq	5.1	cf	30	Q1	Schimmel et al. 1974
Aro-FIS	Atlantic salmon	*Salmo salar*	ELS	Cold	Fresh	195	aq	6	m	5.8	Q2	Fisher et al. 1994
PCB-BLA	Mummichog	*Fundulus heteroclitus*	Adult	Warm	Salt	56	di	6.65	fd	13	Q2	Gutjahr-Gobell et al. 1999
A1254	Pinfish	*Lagodon rhomboids*	ELS	Warm	Salt	14	aq	14	m	66	Q2	Hansen et al. 1971
PCB-BLA	Mummichog	*Fundulus heteroclitus*	Adult	Warm	Salt	40	inj	14.25	ip	54	Q2	Black et al. 1998a
Clo A50	Eurasian minnow	*Phoxinus phoxinus*	Adult	Warm	Fresh	300	di	15	m	28	Q2	Bengtsson 1980
A1254	Common sole	*Solea solea*	ELS	Cold	Salt	40	aq	17.2	li	50	Q2	Foekema et al. 2014
A1254	Spot	*Leiostomus xanthurus*	ELS	Warm	Salt	20	aq	46	m	51	Q3	Hansen et al. 1971
A1254	Brook trout	*Salvelinus fontinalis*	ELS	Cold	Fresh	21	par	77.9	m	92	Q3	Freeman and Idler 1975
A1016	Pinfish	*Lagodon rhomboides*	Adult	Warm	Salt	42	aq	106	m	47	Q3	Hansen et al. 1974
A1248	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	30	aq	120	cf	44	Q3	DeFoe et al. 1978
Aro-MAY	Rainbow trout	*Oncorhynchus mykiss*	ELS	Cold	Fresh	90	aq	120	m	31	Q3	Mayer et al. 1985
A1254	Brook trout	*Salvelinus fontinalis*	ELS	Cold	Fresh	118	aq	125	m	18	Q4	Mauck et al. 1978
A1248	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	30	aq	138	cf	25	Q4	Nebeker et al. 1974
Clo A50	Eurasian minnow	*Phoxinus phoxinus*	ELS	Warm	Fresh	300	par	170	m	83	Q4	Bengtsson 1980
A1016	Sheepshead minnow	*Cyprinodon variegates*	ELS	Warm	Salt	33	aq	200	m	82	Q4	Hansen et al. 1975
Clo A50	Goldfish	*Carassius auratus*	Adult	Warm	Fresh	22	aq	250	m	50	Q4	Hattula and Karlog 1972
A1260	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	30	aq	260	cf	69	Q4	DeFoe et al., 1978 DeFoe, Veith, and Carlson 1978
A1254	Sheepshead minnow	*Cyprinodon variegates*	Adult	Warm	Salt	28	aq	300	cf	95	Q5	Hansen et al. 1973
A1016	Sheepshead minnow	*Cyprinodon variegates*	Adult	Warm	Salt	28	aq	365	cf	91	Q5	Hansen et al. 1975
A1254	Coho salmon	*Oncorhynchus kisutch*	ELS	Cold	Fresh	260	di	645	m	100	Q5	Mayer et al. 1977
A1242	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	240	aq	795	cf	100	Q5	Nebeker et al. 1974
A1016	Sheepshead minnow	*Cyprinodon variegates*	ELS	Warm	Salt	28	aq	1100	m	88	Q5	Hansen et al. 1975
A1254	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	240	aq	2950	cf	100	Q5	Nebeker et al. 1974

[a]Sample met acceptability criteria but was determined to be an outlier and was not included in the analysis.

PCB = mixture of polychlorinated biphenyls (see S1 Table PCB in the Supplemental Data for description); Life stage = adult (reproductive capable); ELS = early life stage including nonreproductive juveniles; Temp. = testing temperature range; Saline = salinity conditions used in testing; Exp (d) = duration of testing in days; Route = route of PCB exposure (di = dietary; inj = injection; aq = aqueous; par = parental transfer); Concn. = PCB tissue concentrations in µg/g in total PCB whole-body wet weight; Conc code = code defining method of determining PCB tissue concentrations (m = direct measure; d = measured dry weight converted; fd = dietary exposure converted; li = measured lipid weight, converted; ip = injected dose converted; cf = concentration determined from study reported bioconcentration factor (see *Materials and Methods* and Supplemental Data, Table S1 for further description); Quint = identifies 20th quintile for each data (Q1 = 0–20; Q2 = 20–40, Q3 = 40–60; Q4 = 60–80; Q5 = 80–100).

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TABLE 2: Growth-related lowest-observed-adverse-effect residue concentration and other critical information from criteria-selected laboratory studies of fish exposed to polychlorinated biphenyls (PCBs)

PCB	Common name	Scientific name	Life stage	Temp.	Saline	Exp (d)	Route	Concn. µg/g	Concn. code	Effect %	Quint	Reference
PCB-ORN	Zebrafish	*Danio rerio*	Adult	Warm	Fresh	91	di	0.14	M	26	Q1	Om et al. 1998
A1254	Atlantic croaker	*Micropogonias undulates*	Adult	Warm	Salt	17	di	2.1	Fd	12	Q1	Thomas and Wofford 1993
PCB-BLA	Mummichog	*Fundulus heteroclitus*	Adult	Warm	Salt	40	inj	2.85	lp	12.5	Q1	Black et al. 1998a
Aro-FIS	Atlantic salmon	*Salmo salar*	ELS	Cold	Fresh	195	aq	3	M	16.9	Q1	Fisher et al. 1994
A1254	Atlantic croaker	*Micropogonias undulates*	ELS	Warm	Salt	13	par	3.2	M	4.1	Q2	McCarthy et al. 2003
PCB-BLA	Mummichog	*Fundulus heteroclitus*	Adult	Warm	Salt	56	di	6.65	Fd	26	Q2	Gutjahr-Gobell et al. 1999
A1242	Channel catfish	*Ictalurus punctatus*	ELS	Warm	Fresh	130	di	14.33	M	39	Q2	Hansen et al. 1976
A1248	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	30	aq	36	Cf	20	Q3	DeFoe et al. 1978
Aro-LEA	Coho salmon	*Oncorhynchus kisutch*	ELS	Cold	Fresh	90	di	43	m[a]	25	Q3	Leatherland and Sonstegard 1978
A1254	Arctic charr	*Salvelinus alpinus*	ELS	Cold	Salt	90	di	50	fd	33	Q3	Jorgensen et al. 2004
K500	Hybrid tilapia	*Oreochromis* sp.	Adult	Warm	Fresh	84	di	50	fd	18	Q3	Shiau and Chen 1992
A1248	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	30	aq	60	cf	50	Q4	Nebeker et al. 1974
A1254	Brook trout	*Salvelinus fontinalis*	ELS	Cold	Fresh	48	aq	71	m	23	Q4	Mauck et al. 1978
A1248	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	30	aq	120	cf	66	Q5	DeFoe et al. 1978
Aro-MAY	Rainbow trout	*Oncorhynchus mykiss*	ELS	Cold	Fresh	90	aq	120	m	11	Q5	Mayer et al. 1985
A1254	Rainbow trout	*Oncorhynchus mykiss*	ELS	Cold	Fresh	365	di	150	fd	39	Q5	Cleland et al. 1988
A1260	Fathead minnow	*Pimephales promelas*	ELS	Warm	Fresh	30	aq	260	cf	30	Q5	DeFoe et al. 1978

[a]Measurement reported in companion study (Leatherland et al. 1979).

PCB = mixture of polychlorinated biphenyls (see SI Table PCB in the Supplemental Data for description); Life stage = adult (reproductive capable); ELS = early life stage including nonreproductive juveniles; Temp. = testing temperature range; Saline = salinity conditions used in testing; Exp (d) = duration of testing in days; Route = route of PCB exposure (di = dietary; inj = injection; aq = aqueous; par = parental transfer); Concn. = PCB tissue concentrations in µg/g in total PCB whole-body wet weight; Concn. code = code defining method of determining PCB tissue concentrations (m = direct measure; d = measured dry weight converted; fd = dietary exposure converted; ip = injected dose converted; cf = concentration determined from study reported bioconcentration factor (see *Materials and Methods* and Supplemental Data, Table S2 for further description); Quint = identifies 20th percentile quintile for each data (Q1 = 0–20; Q2 = 20–40, Q3 = 40–60; Q4 = 60–80; Q5 = 80–100).

Probabilistic sensitivity distributions for MGR

Probabilistic concentration-percentage rank regressions were calculated for the MGR datasets (Figure 3), Developed independently for each dataset, these highly correlated regressions (Supplemental Data, Table S5) were used to generate likelihood of significant effect and concentration predictions (Tables 5 and 6). For example, at a given tissue concentration of 1 µg/g PCB, the regressions predict that 7% of fish species would experience significant mortality, 11% of species would experience adverse growth effects, and 27% of species would experience reproductive impairment (Table 5). As an inverse example, significant MGR in 25% of fish species would occur at PCB tissue concentrations of 7.5, 3.9, and 0.8 µg/g, respectively (Table 6). It would be likely that some, if not all, of these effects would be occurring simultaneously among the most sensitive species (those impacted at the lowest PCB concentrations).

PCB-induced fish mortality dataset meta-analysis

PCB concentration–mortality effects regression. The linear regressions of percentage mortality and PCB tissue concentration had nearly identical slopes for the full dataset and for the quintiles condensed datasets (Supplemental Data, Table S8 and Figure 4). Calculated quintile average datapoints (PCB tissue concentration and percentage mortality pairs; Table 7) were used to generate the quintile regression. For example, the second quintile, Q2 (20–40th percentiles), fell between 5.6 and 23.0 µg/g and contained 6 LOAER values (Table 1, Q2: 6.0, 6.7, 14.0, 14.3, 15.0, and 17.2 µg/g), resulting in a geometric mean PCB tissue concentration of 11.3 µg/g (± 1.0 µg/g SE); the corresponding percentage mortalities (Table 1, Q2: 5.6, 13, 66, 54, 28, *and* 50%, respectively) resulted in a Q2 average percentage mortality of 36.1 ± 9.9%. The log PCB concentration values were used in both regressions because concentration was log-normally distributed. Both regressions were significant ($p \leq 0.05$) and passed the normality ($p > 0.05$) and homogeneous variance ($p > 0.05$) tests. The mortality quintile regression improved the dataset correlation (full: $r^2 = 0.46$ vs quintile: $r^2 = 0.79$; Supplemental Data, Table S8). The expected percentage mortality was calculated across a range of PCB exposure tissue concentrations (Table 8). The quintile regression indicated that mortality would not be expected at or below PCB tissue concentrations of 0.1 µg/g. However, at a concentration of 0.5 µg/g, the quintile regression predicted a mortality of 10%. The quintile regression predicted that mortality would increase by approximately 20% with every order of magnitude increase in exposure concentration. Furthermore, expected PCB exposure concentrations were predicted at a set of given mortality rates (%; Table 9). This provided a way to identify the concentrations associated with expected magnitudes of effect, for example, 10% mortality would be expected at 0.4 µg/g and 25% mortality would be expected at 2 µg/g.

The uncertainty regressions representing a range of effect probabilities were generated (Figure 4) and allowed probability

TABLE 3: Reproductive-related lowest-observed-adverse-effect residue concentrations and other critical information from criteria-selected laboratory studies of adult fish exposed to polychlorinated biphenyls (PCBs)

PCB	Common name	Scientific name	Temp.	Saline	Exp (d)	Route	Concn. µg/g	Concn. code	Effect %	Quint	Endpoint	Reference
PCB-DAU	Zebrafish	*Danio rerio*	Warm	Fresh	60	di	0.26	Fd	26	Q1	↓ Fertilization rate	Daouk et al. 2011
A1260	Barbell	*Barbus barbus*	Warm	Fresh	50	di	0.52	D	52	Q1	↓ Eggs/kg♀	Hugla and Thome 1999
PCB-ORN	Zebrafish	*Danio rerio*	Warm	Fresh	91	di	1.1	M	30	Q2	↓ Eggs/♀	Orn et al. 1998
PCB-BLA	Mummichog	*Fundulus heteroclitus*	Warm	Salt	40	inj	2.85	Ip	60	Q3	↓ Eggs/♀	Black et al. 1998a
Clo A50	Eurasian minnow	*Phoxinus phoxinus*	Warm	Fresh	300	di	15	M	40	Q3	↓ # Spawns	Bengtsson 1980
A1242	Fathead minnow	*Pimephales promelas*	Warm	Fresh	255	aq	107	M	65	Q4	↓ Eggs/♀	Nebeker et al. 1974
Clo A50	Three-spined stickleback	*Gasterosteus aculeatus*	Cold	Fresh	105	di	289	M	69	Q5	↓ # Spawns	Holm et al. 1993
A1254	Fathead minnow	*Pimephales promelas*	Warm	Fresh	255	aq	429	M	58	Q5	↓ eggs/♀	Nebeker et al. 1974

PCB = mixture of polychlorinated biphenyls (see SI Table PCB in the Supplemental Data for description); Temp. = testing temperature range; Saline = salinity conditions used in testing; Exp (d) = duration of testing in days; Route = route of PCB exposure (di = dietary; inj = injection; aq = aqueous). Concn. = PCB tissue concentrations in µg/g in total PCB whole-body wet weight; Concn. code = code defining method of determining PCB tissue concentrations (m = direct measure; d = measured dry weight converted; fd = dietary exposure converted; ip = injected dose converted (see *Materials and Methods* and Supplemental Data, Table S3 for further description); Quint = identifies 20th percentile quintile for each data (Q1 = 0–20; Q2 = 20–40, Q3 = 40–60; Q4 = 60–80; Q5 = 80–100); Endpoint = method used to assess adverse reproductive outcomes.

ranges to be calculated for any PCB tissue concentration (Supplemental Data, Uncertainty). For example, at a 5-µg/g PCB exposure concentration, the predicted 32.5% mortality has a 50% confidence interval (CI) ranging from 26.5 to 38.5% and a 95% CI range from 10.3 to 54.5% mortality.

During the initial regression diagnostics process, one datapoint (0.32 µg/g and 92% mortality; Hugla and Thome 1999) was identified as an outlier (studentized deleted residual value > 3). A preliminary regression including this datapoint predicted substantial mortality at PCB tissue concentrations not supported by any other studies within the dataset. For those reasons, this datapoint was excluded from further analysis and all regressions excluded the outlier.

Evaluation of ancillary variables within PCB mortality dataset

Influence of specific PCB mixtures on mortality. The average LOAER values for PCB mixtures followed the same concentration–response trend as the quintile data (Figure 5A and Supplemental Data, Table Average PCB Mixtures). Regression analysis showed a significant, positive log-linear relationship among PCB mixture concentration–mortality pairs (regression $p = 0.001$; $r^2 = 0.74$), and none of the average PCB mixture values were observed to be an outlier or to be exerting undue influence on the regression. This trend suggested dose–response as an important factor among average PCB mixture datapoints (Figure 5A). However, differences in potency certainly exist among technical mixtures and individual PCB congeners, as established in the literature. Furthermore, due to a paucity of available, appropriate data, mixture-specific LOAER values would be based on 1 or 2 datapoints for most PCB mixtures. For those mixtures with multiple datapoints, individual mortality-based LOAER regressions for both A1254 ($n = 13$) and A1016 ($n = 4$) had positive dose–response relationships (Figure 5B). For A1254 there was a significant log-linear regression ($p = 0.01$; $r^2 = 0.46$), whereas A1016 LOAERs in fish increased with concentration; however, the relationship was not significant ($p = 0.2$ $r^2 = 0.58$), likely due to the small number of studies in the dataset ($n = 4$). The percentage effects associated with the different PCB mixtures were significant. However, no individual pairs of LOAER values for PCB mixtures tested as significantly different (Tukey's test, $p > 0.05$). This may be due to the wide range of mortality responses and the overall influence of PCB concentration on the percentage effect. For example, the A1242 LOAER caused 100% mortality (Nebeker et al. 1974), whereas a study with a mixture of Aroclors caused an 8.2% increase in mortality (Fisher et al. 1994). The strong trend of percentage effect increasing with concentration, observed across the mortality dataset generally, supported our inclusion of all the vetted data, which, in turn, provided better resolution across the spectrum of PCB exposure-related mortalities.

Influence of species tested on mortality. Whereas species average LOAERs varied in both PCB concentration and percentage mortality, the relationship between geomean PCB LOAER

TABLE 4: Metadata and descriptive statistics for vetted mortality, growth, and reproduction lowest-observed-adverse-effect residue concentration datasets

		Mortality	Growth	Reproduction
Data inclusion	Studies	22	15	7
	Data points	29	17	8
	PCB mixes	10	10	7
	Fish species	16	11	6
Life history parameters	Warm/cold water	22/7	11/6	7/1
	Fresh/salt water	16/13	12/5	7/1
	Adult/early life stage	9/20	5/12	8/0
Tissue concentration information (μg/g)	Mean	271	58.4	105.6
	SE	±107	±16.9	±58.3
	Median	106	43.0	8.9
	GeoMean	49	19.3	9.8
	GeoMean SE	±8.8	±4.2	±4.7
	Minimum	0.8	0.14	0.26
	Maximum	2950	260	429
	Normality[a]	Fail ($p < 0.001$)	Pass ($p = 0.063$)	Fail ($p = 0.009$)
	Log-normality[b]	Pass ($p = 0.077$)	Pass ($p > 0.20$)	Pass ($p > 0.20$)
	20th Percentile	5.6	3.0	0.8
	40th Percentile	23.0	23.0	2.5
	60th Percentile	124	50.0	33.4
	80th Percentile	276	110	216
Percentage effect information	Mean	54.7%	26.5%	50.0%
	SE	±4.4%	±3.8%	±5.7%
	Median	50.0%	25.0%	55.0%
	Minimum	5.8%	4.1%	26.0%
	Maximum	100.0%	66.0%	69.0%
	Normality[a]	Pass ($p > 0.20$)	Pass ($p > 0.20$)	Pass ($p > 0.20$)

[a]Normality determined by Kolmogorov–Smirnov with Lilliefors correction.
[b]Tissue concentration data were log-transformed and then retested for normality.
PCB = polychlorinated biphenyl; SE = standard error.

and average percentage mortality for individual species generally followed the same concentration–response trend as the quintile regression (Figure 6A) and was significant ($p = 0.01$, $r^2 = 0.39$). Residual analysis of that regression found no outliers. The geometric mean mortality-based LOAER values in fish spanned 4 orders of magnitude, from 0.75 μg/g for tilapia to 399 μg/g for fathead minnow (Supplemental Data, Table Average Fish Species). Many species occurred singly within the dataset. Species with multiple mortality studies generally had large SEs. The geomean mortality LOAER values for pinfish (38 ± 24 μg/g; $n = 2$), mummichog (9.7 ± 2.3 μg/g; $n = 2$), sheepshead minnow (93 ± 42 μg/g; $n = 6$), and fathead minnow (399 ± 116 μg/g; $n = 5$) had SEs ranging from 20 to 60% of the geomean mortality LOAER value. Species-specific data for both PCB LOAER values and percentage effect failed the normality test and were therefore assessed with an ANOVA of ranked data. No significant differences were observed in either geomean mortality-based PCB tissue LOAER values ($p = 0.24$) or average percentage mortality ($p = 0.78$) among the species evaluated in this dataset.

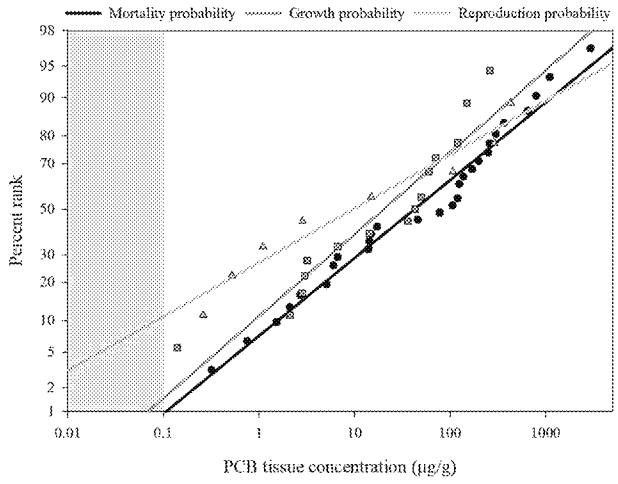

FIGURE 3: Probabilistic sensitivity analyses for mortality (circles, black line), growth (squares, orange line), and reproductive (triangles, cyan line) polychlorinated biphenyl (PCB) tissue lowest-observed-adverse effect residual concentration. Blue area represents tissue concentrations below the lower limit of applicability (0.1 μg/g).

Individual regressions for fathead minnow ($n = 5$) and sheepshead minnow ($n = 6$) showed significantly positive concentration–response data ($p = 0.04$, $r^2 = 0.81$; and $p = 0.002$; $r^2 = 0.92$, respectively; Figure 6B). Slopes of the linear regressions for both were steeper and shifted to the right, relative to the quintile mortality regression, suggesting a more homogeneous response among the populations of fish tested (steeper slope) and lesser sensitivity (shifted right), compared with the all-species regression. However, these datasets were small and confounded by datapoints generated from multiple PCB mixtures (4 for fathead minnow, 2 for sheepshead minnow). Overall, the lack of significant difference suggests that each species represents an overlapping continuum of their individual sensitivities. Again, these analyses suggest the usefulness of an overall approach that is more inclusive of all available data and species.

TABLE 5: Percentage of the fish species predicted to experience significant mortality, growth, and reproductive effects based on the associated polychlorinated biphenyl (PCB) tissue concentration as estimated from the MGR probabilistic sensitivity distribution regression[a]

	Predicted % fish species associated with specified PCB tissue concentration (μg/g)												
Dataset	0.001	0.01	0.05	0.1	0.5	1	5	10	50	100	500	1000	5000
Mortality (%)	0.00	0.06	0.44	0.9	4.2	7.3	20.3	28.7	52.5	63.0	83.1	89.0	96.8
Growth (%)	0.00	0.09	0.70	1.5	6.5	10.9	28.3	38.6	64.4	74.3	90.5	94.5	98.8
Reproduction (%)	0.7	3.2	7.8	10.9	21.2	27.0	42.8	50.2	67.0	73.4	85.5	89.4	95.3

[a]Regression slope and intercept information are found in the Supplemental Data, Table S5. Data do not indicate the degree of effect (percentage effect) but rather the likelihood that a significant effect will occur at a given PCB concentration. Shaded areas are outside the predictive range of the dataset.

Influence of life history variable on mortality. No significant differences were observed among averages of paired test condition variables (warm/cold water, fresh/salt water, adult/ELS). There was a great degree of overlap among PCB LOAER and percentage mortality averages for these data. Enough data were available that concentration–response regressions could be generated for each life history variable set: life stage (adult/ELS; Figure 7A), temperature (warm/cold; Figure 7B), and salinity (fresh/salt water; Figure 7C). Each of these life history variable regressions of the LOAER mortality data was significant ($p < 0.05$). However, the regression slopes and intercepts, and the degree of overlap, suggested that these differences in fish life history did not appear to influence the PCB tissue concentration–mortality relationship. Life history–based paired variable regressions of LOAER values for mortality did not vary substantially from the quintile regression that represented the same data from the entire dataset. Overall, this analysis suggested that when one is deriving mortality-based effects thresholds in fish, there is little support for excluding or separating data based on these life history parameters.

PCB-induced fish growth effects dataset meta-analysis

PCB concentration–growth effects regression. There was minimal difference in the linear regressions between the full growth dataset and the quintile dataset (Figure 8 and Supplemental Data, Table S8). Both regressions passed the normality test ($p \geq 0.05$) and the homogeneous variance test ($p > 0.05$). The full dataset regression approached significance ($p = 0.09$) but was poorly correlated ($r^2 = 0.18$), whereas the quintile dataset regression was significant ($p = 0.04$), with an improved correlation coefficient ($r^2 = 0.80$). The expected percentage growth was calculated across a range of PCB tissue concentrations (Table 8). As PCB tissue exposure concentrations increased, the predicted growth inhibition did not exceed 50% based on the regression. The PCB-induced growth inhibition had an upper limit, that is, only a certain degree of growth inhibition was observed before alternative pathways of toxicity were manifested in the exposed organisms, specifically mortality. This was observed in half of the studies in which both mortality and growth inhibition were monitored (DeFoe et al. 1978; Mayer et al. 1985; Thomas and Wofford 1993; Gutjahr-Gobell et al. 1999). In these studies, once mortality began to occur, individuals were removed from the population being evaluated for effects on growth. Thus the range of percentage growth reductions within individual studies of the present analysis achieved a maximum of 66% (DeFoe et al. 1978; Table 2), and the average percentage growth inhibition in the fifth quintile (Q5) was 37 % (±11%; Table 7). Furthermore, at a certain PCB exposure concentration, the percentage growth inhibition could be predicted. For example, a 25% inhibition of growth was predicted at 12 μg/g (Table 9).

The uncertainty regressions were generated for the growth dataset, representing a range of probabilities (Figure 8) and allowed probability ranges to be calculated for any PCB tissue concentration (Supplemental Data, Uncertainty). For example, at 10 μg/g, the quintile growth regression predicted 24.4% growth inhibition with a 50% CI ranging from 18.2 to 30.5%, and a 95% CI range from 1.2 to 47.5% from the uncertainty regressions.

Evaluation of ancillary variables on the growth dataset

Influence of PCB mixture on growth. A concentration–percentage effect regression of the 10 PCB mixture average datapoints (LOAER concentration–growth effect pairings) was

TABLE 6: Predicted polychlorinated biphenyl (PCB) exposure concentration associated with a given percentage of fish species experiencing significant mortality, growth, and reproductive effects as estimated by the mortality, growth, and reproductive probabilistic sensitivity distribution[a]

	Predicted PCB tissue concentrations (μg/g) associated with specified % fish species with significant effects												
Dataset	5%	10%	20%	25%	30%	40%	50%	60%	70%	75%	80%	90%	95%
Mortality	0.6	1.6	4.9	7.5	11.0	22.1	42.5	81.5	164	241	370	1148	2922
Growth	0.4	0.9	2.6	3.9	5.6	10.9	20.3	37.6	72.9	105	158	463	1125
Reproduction	0.02	0.08	0.43	0.80	1.4	3.8	9.8	25.1	68.7	120	223	1143	4409

[a]The regression slope and intercept information are found in the Supplemental Data, Table S5. Data do not indicate the degree of effect (percentage effect) but rather the likelihood that a significant effect will occur at a given PCB concentration. Shaded areas are outside the predictive range of the dataset.

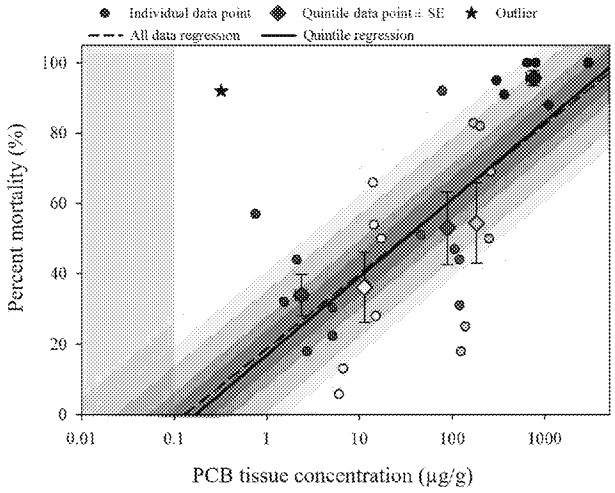

FIGURE 4: Linear regression of mortality (%) and polychlorinated biphenyl (PCB) tissue concentrations (µg/g), for all lowest-observed-adverse effect residual concentration (LOAER) datapoints (●: $n = 29$; dashed line; $p < 0.001$; $r^2 = 0.46$) and for quintile LOAER datapoints (◆: $n = 5$; solid line; $p = 0.05$; $r^2 = 0.75$). Error bars on quintile datapoints represent standard error (SE) values (Table 7). Outlier (star) was excluded from all regressions. Colors represent data from each quintile (Q1 = red; Q2 = yellow; Q3 = pink; Q4 = green, Q5 = blue). Gradient shading represents uncertainty regressions at probabilities 50, 60, 70, 80, 90, 95, and 97.5%. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

not significant (Figure 9A; $p = 0.78$; nonsignificant regression line not shown). The plotted average PCB mixture datapoints relative to the quintile growth regression (Figure 9A) suggested differences. They did not follow the same relative exposure concentration–effect patterns observed in the overall growth regression, with several individual mixtures shifted away from the quintile regression line. No significant differences among mixture average LOAER concentrations ($p = 0.36$) or effects ($p = 0.63$) were observed (Kruskal–Wallis, $p > 0.05$). The majority of PCB mixtures were only tested once within the growth dataset, and those that were tested in multiple studies had highly variable results. The 3 PCB mixtures with multiple study datapoints (A1254, A1248, PCB-BLA) had large SEs for the LOAER concentration (Supplemental Data, Table Average PCB Mixtures). Strong concentration–response trends were observed for A1248 (with 3 datapoints, the geomean concentration was 62 ± 12 µg/g) and A1254 (with 5 datapoints, the geomean was 20 ± 8.5 µg/g; Figure 9B). The regression of A1254 datapoints was similar to the quintile regression. The slope of the A1248 regression was much steeper, but all datapoints in that regression came from one species (fathead minnow), suggesting that these differences did not wholly result from differences in PCB mixtures.

Influence of species on growth dataset. Differences in species average PCB concentration LOAER values for growth inhibition across the 11 different species of fish were substantial, varying by 3 orders of magnitude, but were not statistically significant (Supplemental Data, Table Average Fish Species). Percentage growth effect also varied by species, but variation was not significant. Most species average growth concentration–response datapoints fell near the quintile regression line (Figure 10). The species that were not near the quintile regression (e.g., catfish and zebrafish) represented a single study, but were still within the 70% CI (Figure 8).

Influence of life history variable on growth dataset. No significant differences in average LOAER or percentage growth inhibition were observed for salinity (salt vs fresh) or temperature (warm vs cold). For life stage, a significant difference was observed between the LOAER growth values of ELS fish (78 ± 22 µg/g) and adult fish (12 ± 9.5 µg/g). However, the distribution of the datapoints for growth LOAERs in adult fish overlapped the range of the ELS LOAER values with the exception of one value (Figure 11A). This finding suggests that the significant differences of the average responses may be an artifact of testing approaches (e.g., species tested, PCB mixture, concentrations tested), rather than a true difference. The pattern holds for salinity and temperature variables, for which the individual LOAER values were distributed across the entire range of responses (Figures 11B and C). An analysis comparing individual variable regressions with that of the quintile regression was not conducted, because individual variable regressions were not significant.

TABLE 7: Quintile information derived from mortality, growth, and reproduction datasets (Tables 1–3)[a]

		Mortality effects			Growth effects			Reproduction effects		
Percentile range	Quintiles	Quintile range (µg/g)	Quintile tissue concn. (µg/g ±SE)	Quintile % effect (% ±SE)	Quintile range (µg/g)	Quintile tissue concn. (µg/g ±SE)	Quintile % effect (% ±SE)	Quintile range (µg/g)	Quintile tissue concn. (µg/g ±SE)	Quintile % effect (% ±SE)
0–20%	Q1	0.75–5.6	2.4 ± 0.3	33.9 ± 5.9	0.14–3.0	1.3 ± 0.5	16.6 ± 3	0.26–0.75	0.37 ± 0.08	39 ± 13
20–40%	Q2	5.6–23	11.3 ± 1.0	36.1 ± 9.9	3.0–23	6.7 ± 1.5	23.0 ± 10	0.75–2.5	1.1	30
40–60%	Q3	23–124	88.6 ± 7.8	53.0 ± 10.2	23.0–50.0	44.4 ± 1.7	24 ± 3.3	2.5–33.4	6.5 ± 3.3	50 ± 10
60–80%	Q4	124–276	183 ± 10.8	54.5 ± 10.9	50.0–110	65.3 ± 3.4	36.5 ± 13	33.4–216	107	65
80–100%	Q5	276–2950	753 ± 120	95.7 ± 2.1	110–260	160 ± 14.1	36.5 ± 11	216–429	352 ± 43	63.5 ± 5.5
Normality	*p*		>0.20[b]	0.198		>0.20[b]	>0.20		>0.20[b]	>0.20

[a]Including: the range of polychlorinated biphenyl (PCB) tissue concentration for each quintile, average quintile tissue concentration ± standard error (SE), average quintile percentage effect (±SE), and normality evaluations for quintile datasets.
[b]Normality for quintile tissue concentrations was evaluated using log-transformed values.

TABLE 8: Percentage effects associated with polychlorinated biphenyl (PCB) exposures in fish predicted from linear regression of the full mortality, growth, and reproductive datasets and quintile condensed datasets[a]

	Predicted % effect associated with specified PCB tissue concentration (μg/g)												
Dataset	0.001	0.01	0.05	0.1	0.5	1	5	10	50	100	500	1000	5000
Mortality (%)	—[b]	—[b]	—[b]	—[b]	12.6	18.9	33.7	40.1	54.9	61.2	76.0	82.4	97.2
Quintile mortality (%)	—[b]	—[b]	—[b]	—[b]	10.4	17.0	32.5	39.1	54.6	61.2	76.7	83.3	98.8
Growth (%)	—[b]	1.6	6.9	9.2	14.5	16.8	22.1	24.3	29.6	31.9	37.2	39.5	44.8
Quintile growth (%)	—[b]	—[b]	2.8	5.6	12.2	15.0	21.6	24.4	30.9	33.8	40.3	43.1	49.7
Reproduction (%)	13.3	22.5	28.9	31.7	38.1	40.9	47.3	50.1	56.5	59.3	65.7	68.5	74.9
Quintile reproduction (%)	5.4	16.4	24.1	27.4	35.2	38.5	46.2	49.5	57.2	60.5	68.2	71.6	79.3

[a]For each dataset, slope and intercept data (Supplemental Data, Table S8) were used to predict percentage effect at given PCB tissue concentrations. Values above and below the range of PCB tissue concentrations within the dataset are shown in gray.
[b]Predicted percentage effect <0.

Growth effects in context with other effects. Growth inhibition LOAER values for PCB were influenced by mortality, particularly at higher exposure concentrations. Indeed, no studies had reductions in growth >66% of the control treatments (Table 2). It was likely that PCB-induced growth impacts in fish were limited due to the co-occurrence of mortality at the same exposure concentrations. Because mortality occurred at a given exposure concentration within a study, those individuals that died were not available for assessment of other adverse outcomes, including impairment of growth. Significant growth impacts occurred in several studies at the same exposure concentrations at which significant mortality was also occurring (DeFoe et al. 1978; Mayer et al. 1985; Thomas and Wofford 1993; Gutjahr-Gobell et al. 1999). In some cases, PCB-induced reductions in growth occurred at the next lower exposure concentration, where mortality occurred but was not statistically significant (Nebeker et al. 1974; Mauck et al. 1978; Fisher et al. 1994; Black et al. 1998b; Orn et al. 1998). Growth effects caused by PCBs in fish may also be less prominent due to size-selective mortality. If, as has been suggested, smaller fish are more susceptible to PCBs and die first (Seelye and Mac 1981; Garrido et al. 2015), then the growth (or size) differences between average control and PCB-exposed fish would appear to be not as great, whereas the impact on individual fish (not measured) may be greater. Conversely, when expected mortality was low, there was a greater degree of impact on growth (Tables 1 and 2). This was the case with fathead minnows, a species observed to be among the least sensitive to PCB-induced mortality. Fathead minnows had the greatest degree of growth impairment, 50% and 66% reduction in growth compared to controls (Nebeker et al. 74; Defoe 1974; DeFoe et al. 1978).

The majority of studies reporting effects on growth also monitored and reported other effects occurring concurrently. In the evaluation of A1248 and A1260 (DeFoe et al. 1978), MGR effects were reported. A number of studies measured sublethal effects along with mortality, including physiological effects (Hansen et al. 1976; Mauck et al. 1978; Cleland et al. 1998; Shiau and Chen 1992; Thomas and Wofford 1993), reproductive and physiological effects (Black et al. 1998a; Orn et al. 1998; Gutjahr-Gobell et al. 1999), physiological and immunological responses (Leatherland and Sonstegard 1978; Mayer et al. 1985; Jorgensen et al. 2004), and behavioral responses (Fisher et al. 1994; McCarthy et al. 2003). The PCBs disrupt fish and other organisms through several different pathways and mechanisms of action (Monosson, 2000; Meador et al. 2002). Thus, it was not surprising that most studies reported simultaneous adverse effects occurring at single exposure concentrations of PCBs, including growth inhibition and mortality.

PCB-induced fish reproductive effects dataset meta-analysis

PCB concentration: Reproductive effects regression. The reproduction full and quintile dataset regressions were

TABLE 9: Predicted polychlorinated biphenyl (PCB) exposure concentrations associated with percentage effect responses estimated by linear regression of the full mortality, growth, and reproductive datasets and quintile condensed datasets[a]

	Predicted PCB tissue concentrations (μg/g) associated with specified % effects												
Dataset	5%	10%	20%	25%	30%	40%	50%	60%	70%	75%	80%	90%	95%
Mortality	0.22	0.38	1.12	1.93	3.33	9.90	29.4	87.4	260	447	771	2290	3947
Quintile mortality	0.29	0.49	1.43	2.43	4.13	11.9	34.5	99.9	289	492	836	2420	4116
Growth	0.03	0.13	2.68	12.2	55.7	1161	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Quintile growth	0.09	0.29	3.42	11.7	39.8	464	5406	—[b]	—[b]	—[b]	—[b]	—[b]	—[b]
Reproduction	0.0001	0.0004	0.01	0.02	0.07	0.8	9.8	119	1458	5094	—[b]	—[b]	—[b]
Quintile reproduction	0.001	0.003	0.02	0.06	0.17	1.4	11.1	89.5	722	2051	5825	—[b]	—[b]

[a]For each dataset, slope and intercept data (Supplemental Data Table S8) were used to predict PCB tissue concentration given specified percentage effects. Values above and below the range of PCB tissue concentrations within the dataset shown in gray.
[b]Beyond the scope of theoretical bioaccumulation (>10 000 μg/g).

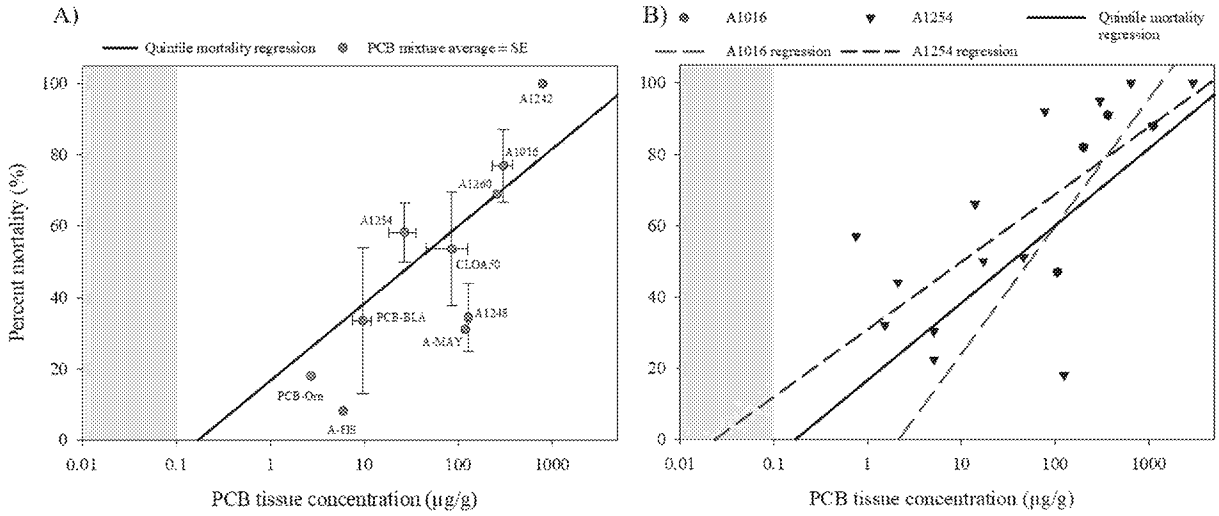

FIGURE 5: Average mortality lowest-observed-adverse effect residual concentration (LOAER) for each polychlorinated biphenyl (PCB) mixture (A) and regressions for 2 mixtures (B; A1254 ▼: $p = 0.01$, $r^2 = 0.46$ [line with shorter dashes]; A1016 ●: $p = 0.2$, $r^2 = 0.58$ [line with longer dashes]) were plotted relative to the quintile mortality regression (solid line). The error bars on PCB mixture LOAER averages (A) represent standard error for PCB concentration and percentage effect. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

similar in slope and intercept (Figure 12 and Supplemental Data, Table S8). Both regressions were significant (full $p = 0.04$; quintile $p = 0.03$) and passed normality and variance testing. The reproduction quintile dataset regression showed a stronger correlation versus the full dataset (full $r^2 = 0.52$; quintile $r^2 = 0.85$). The reproduction regressions were used to predict reproductive effects across all applicable PCB tissue concentrations (Table 8). For example, 39% of fish would have predicted reproductive effects at PCB tissue concentrations of 1 µg/g. Regressions were also used to identify tissue concentrations associated with specific percentage reproductive impairment (Table 9). The reproduction regression predicts effects at a concentration below the level of data applicability of 0.1 µg/g (e.g., 20% loss of reproduction predicted at 0.02 µg/g). However, additional research is needed to support predictions in this range of exposure.

A critical caveat in generating uncertainty regressions for the reproductive dataset (Figure 12) was the small number of reproductive studies ($n = 8$). Only 3 quintiles had sufficient data to calculate the SEs. The uncertainty factors were larger (Supplemental Data, Uncertainty), and the corresponding regressions were wider than the uncertainty estimates for mortality and growth. For example, at 0.5 µg/g, the quintile reproduction regression predicted 35.2% effect with a 50% CI ranging from 27.2 to 42.9%, and a 95% CI ranging from 0 to 76%, using the uncertainty regressions. This broad uncertainty range reflects the limited reproductive data.

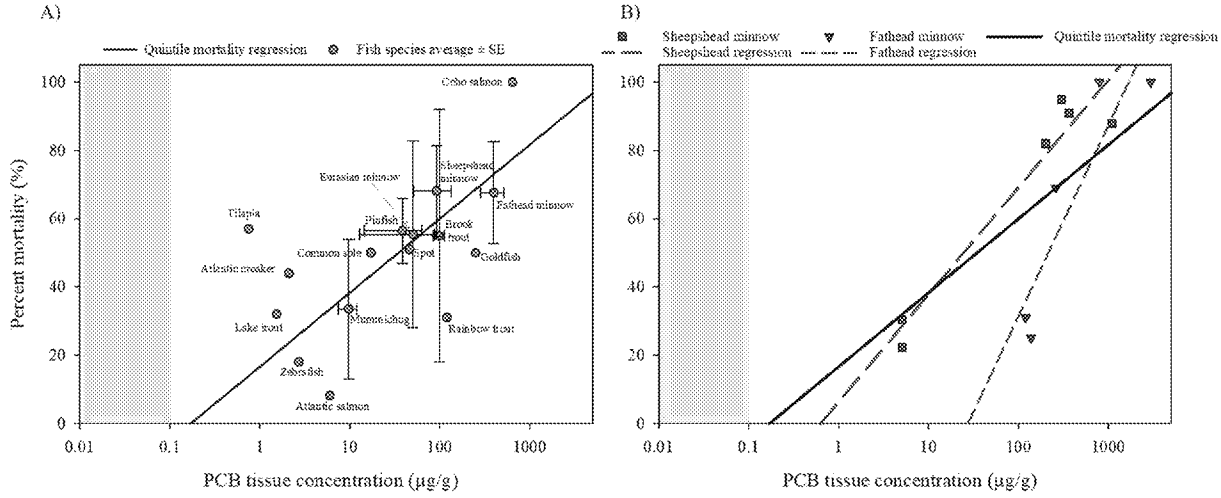

FIGURE 6: Average mortality lowest-observed-adverse effect residual concentration (LOAER) for each individual fish species (A) and regressions for 2 species (B; fathead minnow [pink, line with shorter dashes], $n = 5$; sheepshead minnow [dark green, line with longer dashes], $n = 6$) were plotted relative to the quintile mortality regression (solid line). The error bars on fish species LOAER averages (A) represent standard error for polychlorinated biphenyl (PCB) concentration and percentage effect. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

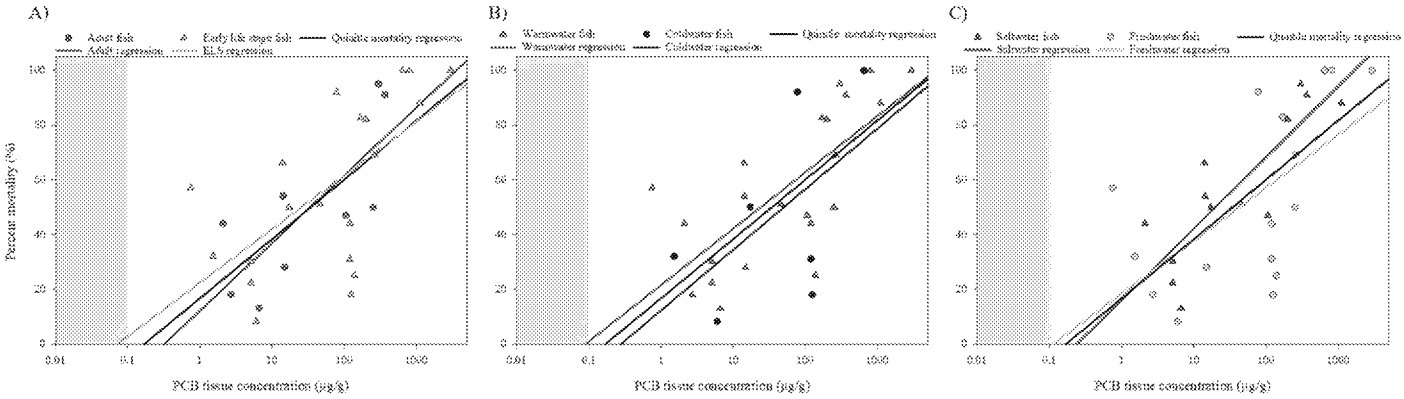

FIGURE 7: Life history variables within the mortality dataset (life stage, temperature, salinity) were evaluated. Percentage mortality–polychlorinated biphenyl (PCB) tissue lowest-observed-adverse effect residual concentration data resulted in significant regressions within each variable: (**A**) life stage (adult vs early life stage [ELS]), (**B**) temperature (warm vs cold), and (**C**) salinity (freshwater vs saltwater). However, little difference was observed between subgroups or relative to the quintile mortality regression (black line). Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

Analysis of the influence of life history characteristics, PCB composition, or other test parameters on reproductive outcomes in fish was not attempted due to the limited number of studies in this dataset. Only 1 of 8 studies was conducted using cold water fish or salt water fish, and only 2 studies used the same PCB mixture. Only 2 species (zebrafish and fathead minnow) were used twice within the 8 included assays. These limitations made it impossible to develop meaningful trends among individual variables (life history or PCB mixture tested). Consequently, the same data-inclusive approach was used with the reproductive effects studies as the evaluation of mortality and growth endpoints.

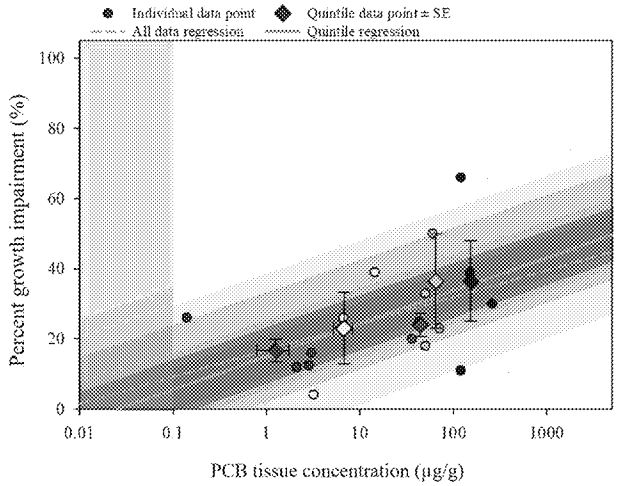

FIGURE 8: Linear regression of growth (%) and polychlorinated biphenyl (PCB) tissue concentrations (µg/g), for all lowest-observed-adverse effect residual concentration (LOAER) datapoints (●: $n = 17$; dashed line; $p = 0.09$; $r^2 = 0.18$) and for quintile LOAER datapoints (◆: $n = 5$; solid line; $p = 0.04$; $r^2 = 0.80$). Error bars on quintile datapoints represent standard error (SE) values (Table 7). Colors represent data from each quintile (Q1 = red; Q2 = yellow; Q3 = pink; Q4 = green; Q5 = blue). Gradient shading represents uncertainty regressions at probabilities of 50, 60, 70, 80, 90, 95, and 97.5%. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

Evaluation of cumulative MGR effects of PCBs on fish

The CLEM dataset regression estimated cumulative effects across the wide range of PCB exposure concentrations in fish (Figure 13). The largest percentage effects (reproduction or mortality + growth) were selected for analysis (Figure 13 and Supplemental Data, Table CLEM). Reproductive data drove the regression at lower PCB concentrations (≤1 µg/g), switching to mortality + growth at greater PCB tissue concentrations. The CLEM-predicted effects were, as expected, greater than any single effect (mortality, growth, or reproduction; Figure 13B). For example, at 1 µg/g PCB concentration, the CLEM would predict 43% cumulative effect compared with 17, 15, and 39% for mortality, growth, and reproduction, respectively (Figure 13B and Supplemental Data, Table CLEM).

Use of field PCB studies to corroborate laboratory-derived PCB effects and regressions

Twenty-four PCB field studies met the inclusion criteria and contained an even distribution of mortality, growth, and reproduction endpoints (Table 10). Egg hatchability was a common endpoint in studies in which wild-exposed fish were brought into the laboratory to evaluate fertilization and hatching relative to maternal PCB exposure. This provided a direct comparison with egg mortality in laboratory studies. There was overlap in the average responses compared with equivalent laboratory results (Figure 14). Furthermore, similar amounts of variation in concentration and effect were observed among both field and laboratory LOAER data.

Overall, PCB-induced mortality in fish from field studies was in line with laboratory-generated mortality data. The average tissue concentration/percentage effect value for field responses fell very near the regression line generated from PCB laboratory mortality quintiles (Figure 14). The majority of field studies showed more severe effects than would be predicted by the laboratory-generated regression line. This finding suggested that the laboratory-generated regression line provided a conservative estimate of PCB-induced mortality (i.e., less than

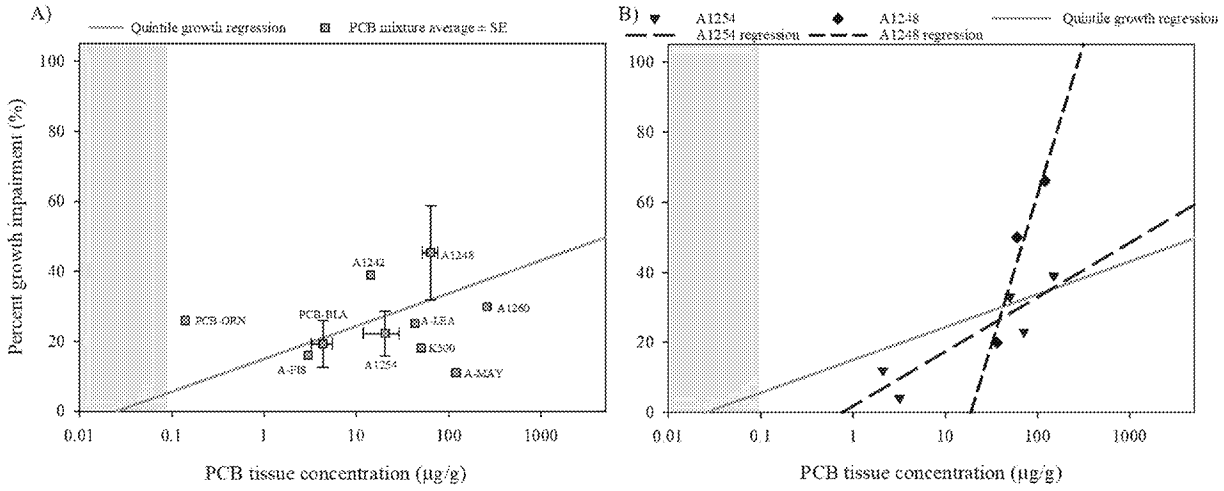

FIGURE 9: Average growth lowest-observed-adverse effect residual concentration (LOAER) for each polychlorinated biphenyl (PCB) mixture (A) and regressions for 2 mixtures (B; A1254 ▼: $p = 0.03$, $r^2 = 0.82$; A1248 ◆: $p = 0.17$ $r^2 = 0.93$) were plotted relative to the quintile mortality regression (solid line). The error bars on PCB mixture LOAER averages (A) represent standard error (SE) for PCB concentration and percentage effect. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

observed in field investigations). The one study with a response substantially below the regression line (Seelye and Mac 1981) was one of a number of lake trout studies (Mac and Schwartz 1992; Mac et al. 1993). When these field lake trout studies were taken together, the average datapoint for lake trout (6.35 µg/g and 25% mortality) fell directly on the regression line. It should also be noted that the range of tissue concentrations from field studies was much narrower and generally lower than those observations in laboratory-based studies with fish. This was likely a functional limitation of field studies, in which the organisms collected only represent survivors. In addition, the migratory nature of fish enhances uncertainty of site fidelity and hence decreases the certainty that a continuous exposure occurred.

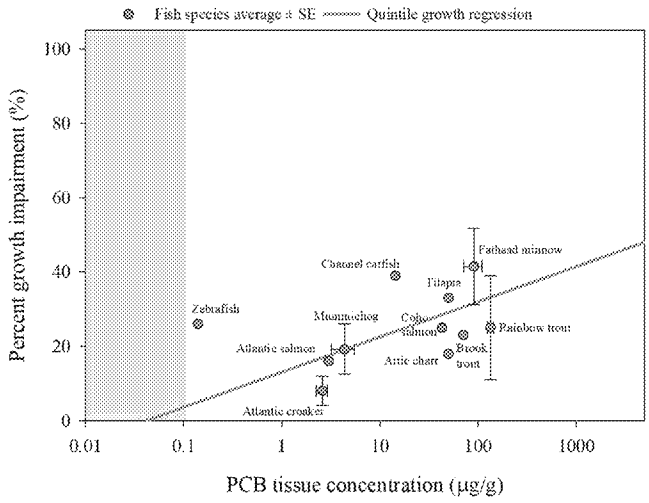

FIGURE 10: Average growth lowest-observed-adverse effect residual concentration (LOAER) for each individual fish species plotted relative to the quintile mortality regression (solid line). The error bars on fish species LOAER averages (A) represent standard error (SE) for polychlorinated biphenyl (PCB) concentration and percentage effect. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g). Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

Growth effects observed in field studies were very similar in magnitude to those observed in laboratory studies at similar measured PCB exposures (Figure 14). The field growth responses were at or near the quintile-derived PCB concentration–growth effect regression. The average value for all fish-PCB field growth studies (9.2 µg/g and 25% growth impairment) fell slightly above that regression line based on laboratory PCB exposure studies (Figures 8 and 14). As with mortality, this finding suggested that the laboratory-derived regression was a conservative approach to assessing growth effects in fish exposed to PCBs under environmental conditions. One caveat with studies from the field would be that growth, even more so than mortality, could be substantially influenced by abiotic and biotic environmental conditions that may not be readily assessed by the routine measurements made in the field ecotoxicological investigations. Thus, if confounding effects of contaminant-induced responses under field conditions were to occur, it would be highly unlikely they would be discovered through routine field investigations. Moreover, any confounding effects would generally tend to indicate increased sensitivity relative to the same exposures in the laboratory.

Unlike mortality and growth studies, the effects on reproduction in adult fish from the field were generally less severe than those observed in the laboratory, based on the 5 field studies evaluated (Table 10 and Figure 14). That is, the reproductive effects associated with PCB exposure measured in field studies were less than those predicted by laboratory-based studies with adult fish. Laboratory reproductive studies focus on fecundity via egg production. Of the 4 field studies below the regression line, 2 measured fertilization rates, 1 counted the number of spawning fish, and 1 evaluated egg weight. Environmental factors confound reproductive effects, influencing the accuracy of the measurements in the field. In addition, reproductive measures in fish can be challenging to assay in fish collected in

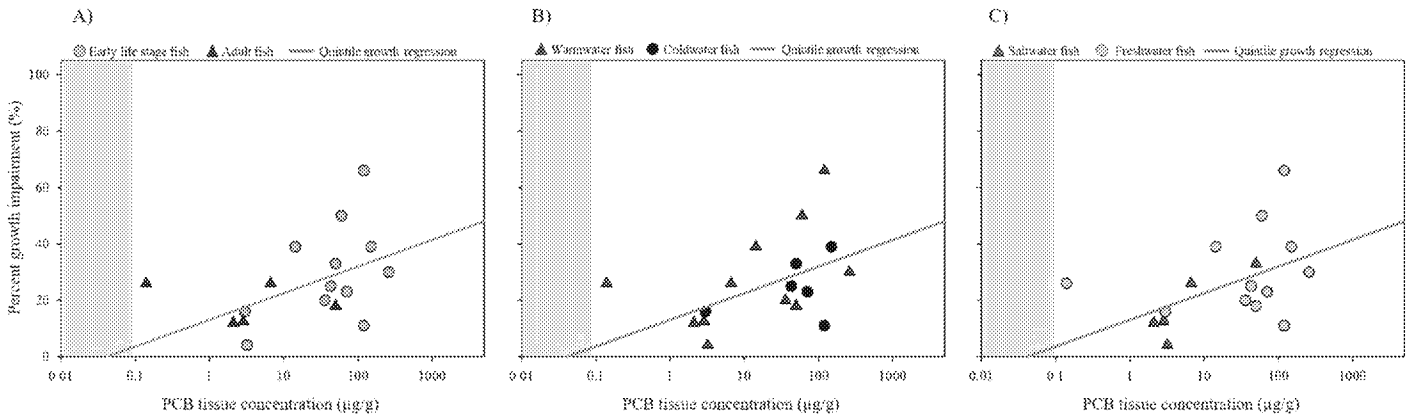

FIGURE 11: Life history variables within the growth dataset (life stage, temperature, salinity) were evaluated. Percentage growth–polychlorinated biphenyl (PCB) tissue lowest-observed-adverse effect residual concentration data were used to calculate linear regressions for the binary subgroups within each variable: (A) life stage (adult vs early life stage), (B) temperature (warm vs cold), and (C) salinity (freshwater vs saltwater). The subgroup datasets did not result in significant regressions. The degree of overlap within the parameters suggests limited differentiation. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

the field, which may also have affected the measurements reported in Table 10.

Many of the PCB-induced physiological, molecular, and cellular responses observed in laboratory studies with fish were also observed in field-based investigations of PCBs. Induction of ethoxyresorufin-O-deethylase (EROD) activity, decreases in vitellogenin, and changes in liver or gonadal somatic indices were all observed in both laboratory and the field studies with fish exposed to PCBs. Although some of these effects were considered adverse, they can be initiated by many different contaminants, not just exposure to PCBs. In addition, the influence of environmental conditions on some of these suborganismal responses leads to highly variable responses of these endpoints. Field studies with the common barbel observed EROD/7-ethoxycoumarin-O-deethylase and cytochrome P450 induction and adverse changes in liver cell histology (Hugla et al. 1995). A follow-up laboratory study with the same species and PCB mixture was able to replicate these same responses, as well as adverse effects in reproduction at the same tissue concentration (Hugla and Thome 1999). The same approach was used for the mummichog, and field and laboratory studies showed similar responses to PCB exposures (Black et al. 1998a, 1998b; Gutjahr-Gobell et al. 1999). Even among these studies, the effects that occur at the physiological, cellular, and molecular level were difficult to scale to organismal level effects. Suborganismal biomarkers of PCB-induced responses in fish are important for development of lines of evidence for causation within risk assessment and injury determination paradigms, as part of an ecoepidemiological framework for determination of causality (Fox 1991) or adverse outcome pathway.

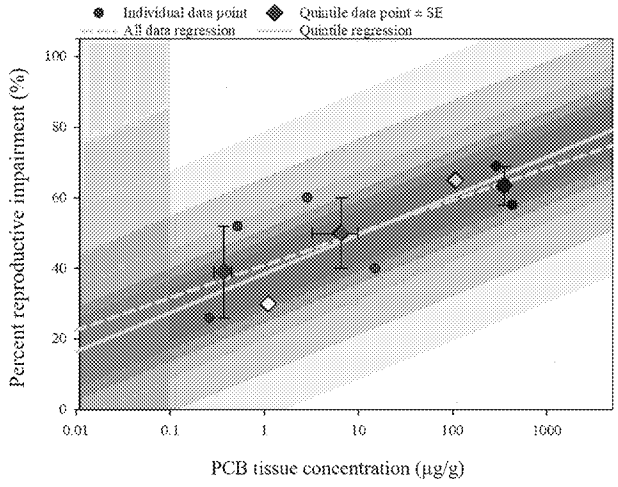

FIGURE 12: Linear regression of reproductive impairment (%) and polychlorinated biphenyl (PCB) tissue concentrations (µg/g), for all lowest-observed-adverse effect residual concentration (LOAER) datapoints (●: $n = 8$; dashed line; $p = 0.04$; $r^2 = 0.46$) and for quintile LOAER datapoints (◆: $n = 5$; solid line; $p = 0.03$; $r^2 = 0.85$). Error bars on quintile datapoints represent standard error (SE) values (Table 7). Colors represent data from each quintile (Q1 = red; Q2 = yellow; Q3 = pink; Q4 = green; Q5 = blue). Gradient shadings represent uncertainty regressions at probabilities 50, 60, 70, 80, 90, 95, and 97.5%. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

DISCUSSION

The available literature on PCB-induced injury in fish populations provided sufficient data for a successful meta-analysis using regressions of PCB-related LOAERs to deliver a unique, data-driven approach to injury assessment. The use of LOAER data, connected to definitive adverse effects, allowed quantitative estimates of PCB tissue concentrations related to MGR injury to fish populations.

The MGR sensitivity distributions (Figure 3 and Tables 5 and 6) provided a means to screen for injury within fish populations and communities. These sensitivity distributions can also provide an initial context to field-derived tissue concentrations. For example, a PCB tissue concentration of 1 µg/g was related to 7% of species within that community experiencing significant

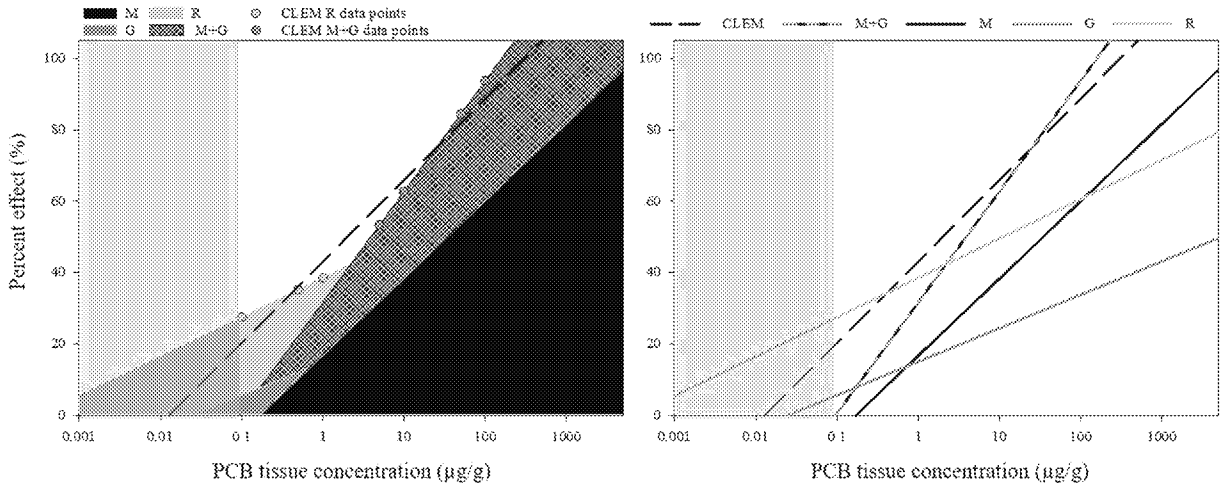

FIGURE 13: A model was developed to estimate the cumulative effect of polychlorinated biphenyls (PCBs) on fish. The largest observed effects between reproduction (cyan) and an additive combination of mortality and growth (M+G; orange hashed) were used to develop datapoints for the cumulative linear regression model (cumulative largest effect model [CLEM]; blue dashed line). The resulting CLEM linear regression provided a means to estimate cumulative effects using the same approach as mortality, growth, and reproduction regression (Supplemental Data, Table CLEM). For comparison, data from the mortality, growth, reproduction (R), and M+G are shown. Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

mortality, and another 11 and 27% could be expected to exhibit reductions in growth and reproduction, respectively. Alternatively, if an effects threshold was set for a fish population, for example 25%, then adverse MGR effects would be expected (according to this criteria) when PCB tissue concentrations were 7.5, 3.9, and 0.8 µg/g, respectively. The actual concentration or effects threshold that would lead to a management action or other regulatory activity would require more site-specific information, based on the needs of regulators and stakeholders, the presence of critical, sensitive, or endangered species, and site-specific exposure analysis.

Application of the individual concentration–effect responses provided the critical connection between tissue concentrations of PCBs and the degree of effect observed in fish. These relationships allow estimation of the specific degree of effect associated with a tissue concentration. As an example, the regressions predict that in a fish population with PCB tissue concentrations of 10 µg/g, 40% of the individuals are likely to have died, and within the remaining individuals, growth would be reduced by 24% and reproduction reduced by 50%. The regression approach also allows simple calculations of estimated injury in fish populations to be developed, based on specific needs of an assessment and site-specific information.

The concentration–effect regressions provided the ability to identify MGR effects across a range of exposures. However, in practical application, ecological risk assessments often develop thresholds based on site-specific criteria, such as the threshold of toxicological concern (TTC; Kroes et al. 2004; Belanger et al. 2015). A TTC is established using a risk value, such as the 5th percentile of the cumulative distribution (de Wolf et al. 2005; Gross et al. 2010; Williams et al. 2011; Mons et al. 2013; Hendriks et al. 2013; Gutsell et al. 2015). In this example, the 5th percentile cumulative distribution for these data would be the point where 5% of species are expected to have mortality, growth, or reproduction effects: at 0.6, 0.4, and 0.02 µg/g PCB tissue concentrations (respectively for mortality, growth, and reproduction; Tables 5 and 6). The corresponding levels of effects anticipated for mortality, growth, and reproduction are 11.8, 11.3, and 19.0%, respectively. Similarly, the 10th percentile TTC corresponds to tissue exposure concentrations of 1.6, 0.9, and 0.08 µg/g with anticipated effects of 21.1, 14.6, and 26.4% for mortality, growth, and reproduction, respectively. This type of approach can be used to estimate MGR effects or exposure based on risk management criteria for protection of fish populations.

The CLEM presented a general, conservative example of how to combine effects, although it was not suggested as a definitive approach to cumulative effects assessment. As with any toxicological model attempting to summarize multiple effects occurring simultaneously, this cumulative effects model approach should be used with caution. Reproductive responses drive the cumulative effects observed at lower concentrations in CLEM. Reproduction is the smallest, and generally the weakest, of the MGR dataset. It is populated with high-quality data, but the limited number of available studies necessarily makes the model less robust than the mortality dataset.

The approach to using these data and tools will depend on the objectives and requirements of the end user. We presented data in several different ways to address varying needs of injury assessment practitioners or other natural resource managers responsible for estimation of effects of PCB exposure to fish populations. The LOAER-based sensitivity distributions for MGR may be used individually. For example, reductions in growth could impact the overwintering success of salmon populations (Quinn and Peterson 1996). The PCB- LOAER growth sensitivity distribution could connect field-measured PCB concentrations in salmon to the likelihood of significant effect, and then to a detailed life history (e.g., overwintering success) to generate the

TABLE 10: lowest-observed-adverse-effect residue concentration values for mortality, growth, and reproductive (MGR) effects from field studies of fish exposed to polychlorinated biphenyls (PCBs) in the environment[a]

Specific PCB or location	Common name	Scientific name	Life stage	Temp.	Saline	MGR	PCB tissue concn. µg/g	% Change	Endpoint	References
New Bedford, MA, USA:	Mummichog	*Fundulus heteroclitus*	Adult	Warm	Salt	M	5.2	23	↑ Mortality	Black et al. 1998b
Measured PCBs: 118, 105, 167, 156, 157, 189, 77, 126, 169			Adult	Warm	Salt	G	2.37	15	↓ Growth	
			Larval	Warm	Salt	M/R	7.3	30	↓ Larval survival	
			Larval	Warm	Salt	M	7.3	20	↑ Larval spine abnormalities	
New Bedford, MA, USA	Winter flounder	*Pseudopleuronectes americanus*	Larval	Warm	Salt	G	9.9	18/6	↓ Weight/length at hatch	Black et al. 1988
						R	3.9	18.9	↓ %Fertilization	
Baltic Sea	Baltic flounder		ELS	Cold	Salt	M	0.21	66	↓ Hatchability	Von Westernhagen et al. 1981
Baltic Sea: A1260[b]	Baltic herring	*Clupea harengus*	ELS	Cold	Salt	M	0.19	38	↓ Hatchability	Hansen et al. 1985
A1242	Rainbow trout	*Oncorhynchus mykiss*	ELS	Cold	Fresh	M	2.7	68	↓ Hatchability	Hogan and Brauhn 1975
San Francisco Bay, CA, USA	Starry flounder	*Platichthys stellatus*	ELS	Cold	Salt	M	2.8	38	↓ Hatchability	Spies and Rice 1988
Newfoundland, CA, USA	Shorthorn sculpin	*Myoxocephalus scorpius*	Adult	Cold	Salt	G	*	14	↑ Lesion	Khan 2011
			Adult	Cold	Salt	O	*	100	↓ Growth	*no direct concentration available
Newfoundland, CA, USA	Winter flounder		Adult	Cold	Salt	G	50	50	↓ Growth	Khan 1999
Housatonic River, MA, USA	Largemouth bass	*Micropterus salmoides*	ELS	Warm	Fresh	M	33	41	↑ Mortality	Tillitt et al. 2001
						G		36	↓ Growth	
Lake Geneva, FR	Artic charr	*Salvelinus alpinus*	ELS	Cold	Fresh	M	0.32	42	↓ Hatchability	Monod 1985
			Adult	Cold	Fresh	R	0.32	14	↓ Fertility	
Puget Sound, WA, USA	English sole	*Parophrys vetulus*	ELS	Cold	Salt	M	2.56	28	↓ Larval survival	Casillas et al. 1991
			Adult			R	3.47	21	↓ %Spawners	
Puget Sound, WA, USA	English sole	*Parophrys vetulus*	Adult	Cold	Salt	R	10	30	↑Fecundity,	Johnson et al. 1997
			ELS			R	10	27	↓ Egg weight	
Clear Creek, IN, USA	Creek chub	*Semotilus atromaculatus*	Adult	Warm	Fresh	G	19.2	21	↓ Growth	Henshel et al. 2006
Oak Ridge, TN, USA: PCB and mercury	Redbreast sunfish	*Lepomis auritus*	Adult	Warm	Fresh	R	1	45	↓ Fecundity	Adams et al. 1989, 1990, 1992
			Adult	Warm	Fresh	G	1	32	↓ Growth	
Mersey Estuary UK: ICES7	European flounder	*Platichthys flesus*	Adult	Cold	Salt	G	0.416	11	↓ Growth	Kleinkauf et al. 2004
Great Lakes, MI, USA	Lake trout	*Salvelinus namaycush*	ELS	Cold	Fresh	M/	2.4	23	↓ Hatchability	Mac and Schwartz 1992

[a]Specific information about each study and selection of values is presented in the Supplemental Data, Table S10 and the Table Field Info.
[b]43 CFR Part 11.62; US Department of the Interior 2009.

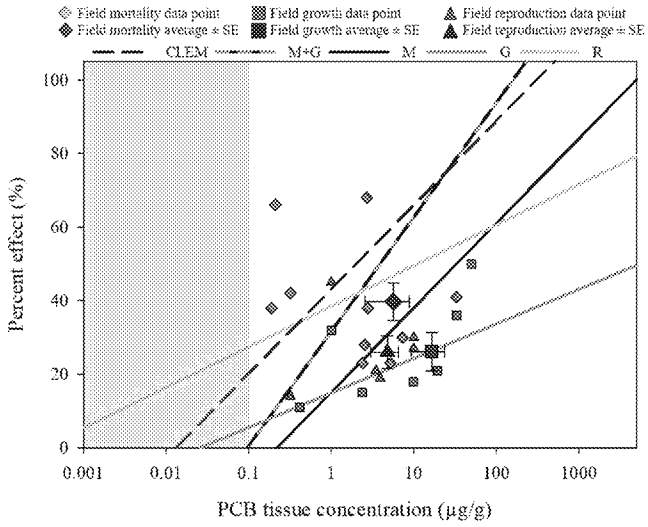

FIGURE 14: Adverse effects measured in fish exposed to polychlorinated biphenyls (PCBs) in the field (◆ = mortality [M]; ■ = growth [G]; ▲ = reproduction [R]) were compared with laboratory-derived fish-PCB adverse effect regressions. The regression lines represent the quintile-derived concentration-effects (mortality, growth, and reproduction), the additive M+G effects, and cumulative response regression (cumulative largest effect model [CLEM]). Individual datapoints for all data are shown. Average responses (larger symbols) for field mortality, growth, and reproduction were calculated (PCB concentration, geometric mean; percentage effect, average) with error bars representing standard error (SE). Blue area represents tissue concentrations below the lower limit of applicability (0.1 µg/g).

associated injury. Uncertainty regressions can be used to determine the bounds of the estimates. The upper bound of the uncertainty regressions could be used to provide a measure of confidence for the protection of species of special concern, like a threatened or endangered species, a species suspected of being particularly sensitive, or an ecologically or economically important species.

Although it was based on the best available data, the meta-analysis we conducted had inherent uncertainties built in to the calculations and LOAER estimates, based on limitations of the available data. Potency differences among the PCB mixtures were perhaps the greatest concern related to the uncertainty incorporated into the meta-analysis. Different PCB congeners and technical mixtures are without doubt more potent than others, particularly those with higher percentages of the dioxin-like PCBs (77, 81, 126, and 169; Safe 1994; Giesy and Kannan 1998; Van den Berg et al. 1998; Kannan et al. 2000; Burkhard and Lukasewycz 2008); nonetheless, the LOAER regressions using total PCB concentrations were robust enough across PCB mixtures to be useful with calculated degrees of uncertainty. The potential for various fish species to exhibit differential sensitivities also added uncertainty. Fish exhibit differing sensitivities to dioxins (Elonen et al. 1998; Tillitt et al. 2017), and differences among species in sensitivity to PCBs were evident in the data we compiled. Although the data in our analysis were derived from studies utilizing 21 species of fish, this represents only a small fraction of the diversity of fish species. There was also uncertainty surrounding the tests themselves, particularly the data from growth studies. due to the confounding effects of PCB-induced mortality that occurred simultaneously as growth effects. Another uncertainty comes from the changes in analytical detection methods and chemical quantification of PCBs that have occurred over the 5-decade time span represented within the dataset. During that time, significant improvements in analytical methods for the detection of PCBs occurred (Subedi and Usenko 2012). A more subtle difference among the data reported from different studies may have been in the degree or extent of statistical analysis, with more recent studies tending toward more statistically driven analysis. Lastly, the datasets used to generate the threshold response regressions were relatively small, particularly for growth ($n = 17$) and reproduction ($n = 8$). Small datasets are not ideal; however, the size of the dataset does not invalidate the analysis or the approach taken. Similarly, small datasets are considered valid when regulatory assessments are developed for Registration, Evaluation, Authorisation, and Restriction (REACH) in Europe (European Chemicals Agency 2008), water quality criteria in the United States (Stephan et al. 1985), and similar assessments in other countries (Belanger et al. 2017). Although it is critical to acknowledge these uncertainties, it is also important to understand that the data used to generate our meta-analysis were screened to meet select criteria, so as to include only the best available information.

A major advantage of this approach was the degree of transparency afforded in the data selection and analysis. Selection criteria provided a clear path to sorting through the fish PCB toxicity data appropriate to support fish injury assessments. The regressions were purposely kept as linear regressions, with defined equations (Equations 8 and 9) used to generate estimates of effect or exposure concentration. Exposure or effects thresholds can be quickly generated based on specific criteria for a fish injury assessment. The approach also allows for updating and is fully adaptable. As additional MGR data become available, they can easily be incorporated into the existing dataset.

The analyses based on the population-level MGR effects provide an anchoring point for relating tissue concentrations to percentage effects. Other effects endpoints, at the physiological, cellular, biochemical, and genetic levels of organization, can be connected and scaled to these MGR anchor points, following the adverse outcome pathway approach (Ankley et al. 2010; Hutchinson et al. 2013). As mechanistic models of toxicity of PCBs are further developed and key events become identified, the same approach, from data vetting to concentration–response regression, could also be applied. This same meta-analysis approach could also be adapted to other pollutants or pollutant classes (e.g., metals, oil, other legacy pollutants). A similar structural framework could be followed: gather data—develop criteria —vet the data—develop effect-specific regression—identify/analyze testing parameters—establish data-driven thresholds and uncertainty factors.

The approaches we describe focused on injury assessment in fish populations exposed to PCBs. Retrospective

assessments, related to the discharge of PCBs into an environment, often require an evaluation of the literature on concentration—adverse effect relationships to help define an injury assessment. Quality data on lethal and sublethal effects, linked to concentrations, provide practitioners with the tools to assess the injuries that may have already occurred within fish populations. This was the purpose of selecting LOAER concentration–effect values from all the existing literature on PCB-induced responses in fish. At the LOAER exposure concentrations in each of the studies examined, a significant adverse effect occurred on mortality, growth, or reproduction. The purpose of the present meta-analysis was the development of an approach to facilitate injury assessments; however, the meta-analysis could also aid in the development of risk assessments. The inclusion criteria provided transparency and the scientific justification behind the data review and selection process. The use of concentration–response regressions provided a data-driven means to estimate PCB effects across the broadest range of concentrations. The approaches we have outlined create a framework for regulators and scientists to utilize the entire array of available data for the purposes of environmental injury assessments of PCB-induced effects on fish populations.

CONCLUSIONS

Our goal was to evaluate PCB-induced adverse effects data in fish and develop tissue concentration–based effects thresholds that provide scientific support to risk and injury assessments. Our study focused on the endpoints survival, growth, and reproduction, those that are most often used by resource managers and risk assessors tasked with prospective and retrospective analyses of the impact of PCB exposures on fish populations.

- This meta-analysis began with clear, transparent data selection criteria to assure use of the best available data.
- The analysis used LOAER to connect PCB exposure concentrations to definitive adverse effects on MGR.
- Sufficient high-quality data was available for this meta-analysis; 55 LOAER datapoints (M 30, G 17, R 8) across 31 studies that met criteria for inclusion.
- An applicability limit of 0.1 μg/g total PCB tissue concentration was established, below which MGR effects were not supported by the available data.
- Sensitivity distributions identified the probability of adverse effects in a population or community, for example, at 7.5 μg/g PCB in tissues, 25% of fish species would be impacted.
- Regressions connected PCB concentrations with the degree of adverse effect expected, for example 1 μg/g PCB in tissues would result in 17% increase in mortality, 15% inhibition of growth, and 39% inhibition of reproduction.
- LOAER regressions using the exposure metric of total PCB concentrations were sufficiently robust across different PCB mixtures to be useful for injury assessment of MGR in fish populations.
- This approach provides a framework to utilize the entire array of available data to assess PCB-induced environmental injury.

The present meta-analysis provides a tool that can be directly applied to injury assessment of fish populations exposed to PCBs. Application of the LOAER regression toxicity threshold values will be dependent on the specific objectives of different natural resource managers; however, the analysis and approach provide an important step forward for evaluation of PCB-related effects on fish populations across a range of environmental exposures.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4335.

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Disclaimer—Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the US Government.

Data Accessibility—Data, associated metadata, and calculation tools not in the Supplemental Data are available from the corresponding author (dtillitt@usgs.gov).

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